The Journal of HEREDITY

KARYOTYPE ANALYSIS IN A SPECIES OF *ORNITHOGALUM*

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Notched Eared Dairy Cattle

Cytogenetics of a Grape Variety

The Cytology of Two Cotton Hybrids

Sex of Parthenogenetic Turkey Embryos

Mitotic Chromosomes of the Domestic Fowl

Genetic Relationship and Reaction to Skin Grafts

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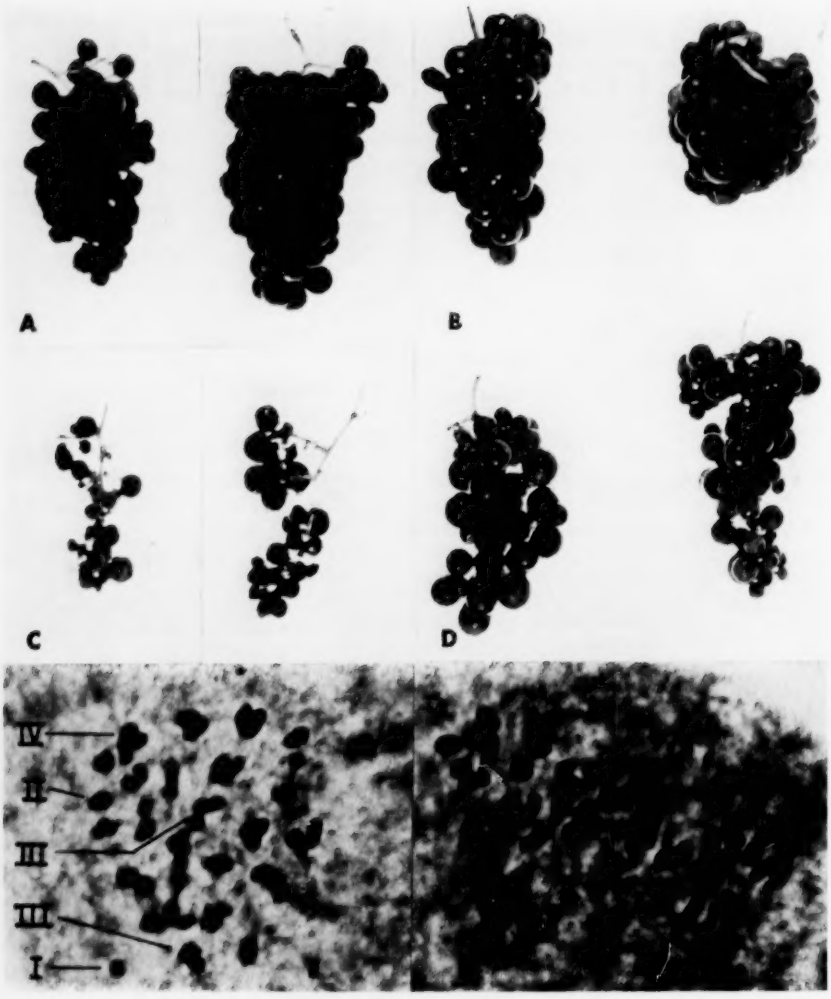

AUTOTETRAPLOID GRAPES

Figure 1

A—Seedling J 17-86. Clusters of F_1 (Zinfandel 4*n*) selfed, from vine showing high fertility. *B*—Seedling J 19-6. Clusters of (Zinfandel 4*n*) × (Folle blanche 4*n*), from vine showing high fertility. *C*—Seedling J 17-83. Clusters of F_1 (Zinfandel 4*n*) selfed, from vine showing low fertility. *D*—Seedling J 19-3. Clusters of (Zinfandel 4*n*) × (Folle blanche 4*n*) from vine showing low fertility. *A-D*, 1/3 ×. *E*—Photomicrograph of a metaphase plate from seedling of (Zinfandel 4*n*) having low fertility; 20 bivalents + 7 quadrivalents + 2 trivalents + 2 univalents. *F*—Photomicrograph of a metaphase plate from seedling of (Zinfandel 4*n*) × (Folle blanche 4*n*) having low fertility; 18 bivalents + 8 quadrivalents + 2 trivalents + 2 univalents. *E* and *F*, 2,500 ×.

CYTOGENETICS OF VITIS

II. Chromosome Behavior and the Fertility of Some Autotetraploid Derivatives of *Vitis vinifera* L.

CURTIS J. ALLEY*

BIOLETTI[2] and Olmo[16] reported the spontaneous occurrence of autotetraploid forms in several *vinifera* grape varieties in California vineyards. Although the resulting berries were larger, a very desirable economic characteristic in table grapes, the vines had certain undesirable features: brittle shoots and clusters, irregular fruit setting, poor leaf coverage, and lower total growth and yield than those of diploid parental clones. Such giant forms remained vineyard curiosities until it was discovered that certain commercial varieties producing large berries in Japan, Europe, and California were actually autotetraploid forms of well-known diploid varieties[16]. This finding aroused widespread interest in the possible breeding of new varieties on the tetraploid level.

The low gametic fertility found in many autotetraploid plants is often attributed to competitive chromosome pairing and irregular distribution during meiosis[5,9,12]. This interpretation caused an early lack of interest in the breeding of new autopolyploid forms, especially when sexual seed production was a necessary precursor for fruit development. For *Vitis*, Olmo[16] suggested that genetic differences were probably more important than meiotic irregularities brought about by the chromosome doubling *per se*, since some autotetraploid varieties were as fertile as the diploid. Secondly, hybridization and selection between autotetraploid varieties produced vines with a wide range in fertility, further supporting the importance of genotypic control. The present study was made to compare meiotic chromosome behavior during microsporogenesis with the fertility of different autotetraploid clones on the basis of the functioning of ovules in forming mature seed and the ability of the pollen to effect fertilization of the ovule. None of the varieties are known to form seed apomictically; fertilization of the ovule, and subsequent development of the endosperm, is necessary in the development of hard seed-coats.

Material and Methods

The diploid clones of the Cornichon, Folle blanche, and Zinfandel varieties used in this work are typical of *Vitis vinifera* L. Corresponding autotetraploid forms had arisen as spontaneous somatic mutations of the diploids and have been propagated since by buds or cuttings. In 1938, Randall[17] made crosses between the autotetraploids Zinfandel × Cornichon, and Zinfandel × Folle blanche, as well as selfing the Zinfandel autotetraploid.

Annual-yield records of the seedling progenies varied markedly from vine to vine. Flower clusters of the parent vines and 260 seedling vines were enclosed in manila paper bags before blossom. At harvest time the berries, both seeded and seedless, were counted and the seeds were extracted and dried. The number of fallen flowers collected in the bag, added to the number of berries gave an exact record of the original number of flowers. Bagging was repeated during four seasons, 1947-1950. Since some vines produced fruit parthenocarpically, "ovule set" rather than berry set was used to classify the vines as of low, medium, or high fertility. With few exceptions each flower has four ovules, and the percentage of these that matured into hard seeds appeared to be a good measure of female gametic fertility.

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From four to six vines; some highly self-fertile and others of low or medium self fertility, were then selected from each of the populations for meiotic chromosome studies.

The chromosome numbers in the root tips of the various parental vines and the autotetraploid derivatives were determined by root tip smears and by paraffin sections, stained with iodine-crystal violet and mounted.

Flower buds collected for meiotic study of microsporogenesis were killed and fixed in a 2:1:1 mixture of chloroform, acetic acid, and ethyl alcohol. The material, stored at 15°F. remained good for more than a year. Temporary aceto-carmine smears were used almost exclusively.

Pollen-germination tests were made using a hanging drop of 20 percent sucrose solution in enclosed cells. When necessary, pollen samples were stored according to the technique described by Olmo[15].

The proportion of ovules functioning in the tetraploids was determined after emasculating the flowers and pollinating with *V. rupestris* St. George, a diploid having highly viable pollen. Flower clusters of the parental diploid and autotetraploid vines and derivatives were emasculated prior to opening. Pollen was applied once, immediately after emasculation, and the clusters were bagged. Emasculated clusters not pollinated served as checks.

The relative viability of the tetraploid pollen was checked by applying it to the male-sterile *V. vinifera* Humisa. Clusters were bagged before blossom. When nearly all the calyptras had been shed the bag was removed and the remaining unopened flowers were removed. The open flowers were pollinated once and the bag was replaced. No attempt was made to limit the amount of pollen applied. Bagged clusters not pollinated served as checks. The latter produced no seeded berries.

Results

Self-fertility of parents and seedlings

Table I indicates that the autotetraploid parental varieties Folle blanche and Cornichon were nearly as self-fertile as their respective diploid forms; whereas the autotetraploid Zinfandel was lower in self-fertility than the diploid. Vines having a high relative self-fertility maintained this high fertilty from year to year, and vines that showed a low fertility were consistently low. Some of the hybrid seedlings showed higher self-fertility than the inbred seedlings.

F_1 seedlings were selected from the inbred and hybrid populations that showed the most significant differences in self-fertility. Vines having a self-fertility of less than five percent were arbitrarily rated as low, from 5 to 11 percent as medium, and above 11 percent as high. The fertility of these seedlings ranged from 1.0 percent (J 19-11) to 28.9 percent (J 19-6), thus exceeding in both directions the fertilities of the parental varieties.

Vines of low self-fertility develop parthenocarpic berries more frequently than do vines of high fertility. For example, vines J 17-90 and J 17-83 are rated low in self-fertility and show a relatively high berry set (44.0 and 25.2 percent, respectively) but a relatively low seeded berry set (8.3 and 4.8 percent, respectively).

Functional pollen of the tetraploids

The ability of the male-sterile diploid variety Humisa to set seed when pollinated by the various diploid and autotetraploid parental vines as well as by their hybrid and inbred derivatives is presented in Table II.

Pollen functioned as well from vines of low self-fertility as from vines of high self-fertility. Pollen of Cornichon 4*n* gave a significantly lower set than did the pollen of Cornichon 2*n*. Apparently, on diploid styles haploid pollen appears to function more readily than does diploid pollen. There is a very high percentage of "floaters"—hollow seeds that are partly lacking in endosperm and, when planted, rarely germinate when the autotetraploid is used as the pollen parent[14].

Functional ovules of the tetraploids

The ability of the various autotetraploid parental vines and derivatives to set seed when outcrossed with haploid pollen was tested using the diploid *V. rupestris* St. George. Results shown in Table II indicate a high correlation between the self-fertility of the vine and the ovule-fertility as tested with haploid pollen. Those vines showing high self-fertility have also a high percentage of functional ovules when outcrossed. Hybrid seedlings of low self-fertility tend to have a greater number of "floaters" than those of high fertility. Inbred seedlings do not show this trend. The percentage of "floaters" is reduced when the autotetraploid is used as the female parent.

Pollen germination

Germination tests were made after the pollination season. Germination of freshly gathered pollen is considerably higher than indi-

cated by these results. The present figures are for pollen that has aged from one to three weeks at room temperature, 70-75° F. Table I indicates that germination of pollen from diploid vines was much higher than pollen from tetraploid vines. No significant differences in germination could be demonstrated between vines of the different classes of self-fertility. Hybrid seedlings tend to show a higher germination than inbred seedlings.

Chromosome pairing at metaphase I

The meiotic chromosomes of *Vitis vinifera* are approximately one μ in length at metaphase I. With practice it is possible to distinguish between univalents, bivalents, trivalents, and quadrivalents. Multivalents greater than quadrivalents are recognized only by irregularities in lobing and by volumetric relationships, so considerable error may be involved in their determination. Usually quadrivalents are closed rings. Since satisfactory smears were not easily obtainable there was a variation in the numbers of pollen-mother-cells analyzed for each seedling. A total of 316 P.M.C.'s were analyzed completely—93 in the parental vines, 160 in one hybrid population, and 63 in one inbred population.

Occasionally quadrivalents are present in diploid varieties, a fact not reported previously. Since Folle blanche *2n* had two univalents and no trivalents, the univalents could have occurred by the failure to synapse or precocious separation of a bivalent. Cornichon *2n* sometimes exhibits a univalent and a trivalent. As only five P.M.C.'s of Cornichon *2n* were examined, the data are not conclusive as to relative frequency of this aberration.

The autotetraploid varieties averaged six to seven quadrivalents, one to two trivalents, and one to three univalents per cell. Univalents were highest in Zinfandel, and lowest in Cornichon. There were no multivalents greater than quadrivalents. Since the frequency of univalents was greater than that of trivalents, results would infer an early separation of bivalents. The autotetraploid varieties had more bivalents than 19, the haploid number.

The autotetraploid derivatives showed no great differences between vines of high and low fertility in the type of chromosome asso-

TABLE I. Self-fertility and pollen germination of parental varieties and selected F_1 seedlings 1947-1950

Variety or seedling no.	Somatic no.	Total no. flowers	Percent berry set	Percent seeded berry set	Percent ovule-set	Fertility rating	Total pollen grains counted	Percent germination
Parental Vines								
Folle blanche	*2n*	8808	32.1	29.5	9.4	medium	10718	12.8
Folle blanche	*4n*	5774	32.1	25.5	9.1	medium	5510	1.0
Zinfandel	*2n*	10113	38.2	33.0	11.3	high	8822	5.3
Zinfandel	*4n*	13389	18.4	15.7	4.6	low	7790	1.5
Cornichon	*2n*	16213	11.3	10.3	6.1	medium	8019	21.8
Cornichon	*4n*	5238	14.5	14.2	6.1	medium	4841	5.0
St. George	*2n*						7497	28.5
Seedlings of Zinfandel *4n* Selfed								
J 17-89	*4n*	2394	48.0	44.4	14.0	high	8101	1.1
J 17-86	*4n*	2239	42.8	40.0	12.4	high	10508	2.2
J 17-90	*4n*	6185	44.0	8.3	2.2	low	9421	1.3
J 17-83	*4n*	4754	25.2	4.8	1.3	low	9404	1.5
Seedlings of (Zinfandel *4n*) × (Cornichon *4n*)								
J 18-48	*4n*	2077	47.0	46.8	20.7	high	6631	3.9
J 18-28	*4n*	1479	48.7	48.5	19.2	high	5177	15.8
J 18-75	*4n*	1426	38.3	36.3	13.0	high	4333	12.0
J 18-90	*4n*	9205	13.3	6.5	1.8	low	7534	16.8
Seedlings of (Zinfandel *4n*) × (Folle blanche *4n*)								
J 19-6	*4n*	3354	67.9	67.6	28.9	high	8863	4.5
J 19-5	*4n*	2986	63.9	63.5	26.8	high	7226	4.8
J 19-7	*4n*	1843	46.6	46.2	16.2	high	5470	3.1
J 19-8	$4n+3$	2186	32.1	31.1	10.4	medium	5642	0.8
J 19-3	*4n*	4701	24.3	16.2	4.3	low	7295	3.7
J 19-11	*4n*	1617	44.2	14.1	1.0	low	2983	2.2

ciation at metaphase I. Vines of the high self-fertility class show higher mean frequencies of trivalents, quadrivalents, multivalents and a lower frequency of bivalents than vines of the low self-fertility class. The vines of the high fertility class show a higher mean frequency only of quadrivalents and multivalents than any of the autotetraploid parental varieties. Inbred vines showed a tendency toward higher mean frequency of univalents and bivalents and a lower mean frequency of trivalents, quadrivalents and multivalents than hybrid vines. Mitotic chromosome counts confirmed the tetraploid nature of the vines as determined by meiotic chromosome pairing.

Unoriented chromosomes

Total chromosome counts could not be made in many P.M.C.'s, because of the clumping and overlapping of groups of chromosomes. Unoriented chromosomes are seen in the cytoplasm off the metaphase; in later stages of meiosis they are apart from the two main groups of chromosomes. Often these unoriented chromosomes are excluded from the daughter nuclei. P.M.C.'s that showed no chromosomes separated from main groups were classified as cells appearing normal. The data for anaphase I and telophase I were combined (Table IV). During anaphase, chromosomes are seen to leave the metaphase plate and progress towards the two poles. Often several chromosomes will lag behind and form a small group. At telophase, chromosomes are seen in two main groups, and isolated from these may be one to several small bodies, each composed of one or more chromsomes. These may divide later, forming two smaller groups, or may become reconstituted into a single group.

Anaphase I—Telophase I

Table IV shows that the parental autotetraploid Cornichon was most regular in chromosome behavior and Zinfandel the most irregular. One bridge was observed in Folle blanche. Vines of the high fertility class indicated a higher percentage of normal P.M.C.'s than the vines of the low fertility class.

Metaphase II

Cornichon again had the highest regularity

TABLE II. Summary of fuctional pollen and functional ovules, 1949-1950

Variety or Seedling No.	Fertility rating	Functional Pollen: Total flowers pollinated	Functional Pollen: Percent berry set	Functional Pollen: Percent seeded berry set	Functional Pollen: Percent ovule set	Functional Pollen: Percent floaters	Functional Ovules: Total flowers emasculated	Functional Ovules: Percent berry set	Functional Ovules: Percent seeded berry set	Functional Ovules: Percent ovule set	Functional Ovules: Percent floaters
Parental Varieties											
Folle blanche $4n$	medium	3611	21.6	6.9	1.9		1399	19.4	17.8	5.0	46.5
Cornichon $2n$		719	36.0	27.0	11.6	16.5					
Cornichon $4n$	medium	3046	27.5	8.6	2.5	94.8	2174	8.7	7.5	2.2	95.2
Zinfandel $2n$		1137	10.9	9.7	3.7	85.0					
Zinfandel $4n$	low	2917	29.5	5.2	1.6	85.1	1357	4.6	4.5	1.2	61.5
St. George $2n$		1411	17.2	12.1	3.2	36.1					
F_1 Seedlings of Zinfandel $4n$ Selfed											
J 17-89	high	2027	26.2	21.5	8.3	82.2	649	26.8	13.6	3.9	62.0
J 17-86	high	3464	25.1	11.5	3.6	84.5	871	9.2	5.2	1.4	70.0
J 17-90	low	2098	20.1	13.9	3.6	62.2	2458	36.0	1.8	0.5	60.9
J 17-83	low	4043	22.8	8.2	2.6	88.7	900	6.1	1.3	0.4	69.2
F_1 Seedlings of (Zinfandel $4n$) × (Cornichon $4n$)											
J 18-48	high	2988	22.7	5.0	2.4	90.5	690	7.8	7.4	2.1	55.2
J 18-28	high	3288	23.3	19.8	7.6	92.0	630	6.8	6.6	2.0	63.3
J 18-75	high	3282	27.9	17.0	5.5	95.0	705	0.6	0.6	0.2	100.0
J 18-90	low	6233	20.4	15.4	5.4	93.0	1959	0.3	0.3	0.1	83.3
F_1 Seedlings of (Zinfandel $4n$) × (Folle blanche $4n$)											
J 19-6	high	4407	24.8	16.7	7.2	89.4	1655	42.5	40.5	15.5	41.6
J 19-5	high	3145	16.9	13.5	4.8	93.2	1384	31.0	30.7	11.3	24.4
J 19-7	high	3335	25.1	9.1	4.4	77.0	677	26.1	26.1	9.2	30.4
J 19-3	low	3714	23.8	12.7	4.1	86.9	1931	22.0	11.6	3.3	60.8
J 19-8	medium	5321	16.1	9.2	2.5	96.8	1062	5.3	5.1	1.6	68.2
J 19-11	low	2660	17.2	10.0	3.0	77.6	798	17.9	0.8	0.2	83.3

and Zinfandel was the most irregular. Vines of the high-fertility class showed a greater degree of irregularity than did vines of the low-fertility class, a reversal of the condition observed at anaphase I-telophase I. Within each class of fertility the hybrid vines had a higher mean percentage of normal P.M.C.'s than the inbred vines.

Anaphase II—Telophase II

Folle blanche had the highest percentage of normal P.M.C.'s, and Zinfandel the lowest. Folle blanche and Cornichon showed no irregularities other than univalents whereas Zinfandel had other irregularities as small unoriented groups or groups and univalents. The high-fertility class showed a greater irregularity than the low-fertility class.

From these results it seems apparent that self-fertility must be largely governed by something other than competition in pairing and chromosome distribution at later stages of meiosis.

Discussion

One of the striking features of *Vitis* is the very low ovule or self-fertility of the diploid plant, often below 50 percent, as compared to the fertility of other genera, which are often above the 75 percent level. This fact should be recognized before further complicating the picture with tetraploidy.

This study of the fertility of the three autotetraploid varieties and their F_1 derivatives has shown that distinct differences exist in self-fertility. Part of this may be due to chromosme behavior at metaphase I and the subsequent unequal distribution of chromosomes, however, this may be only a minor factor. At metaphase I there are no marked differences in chromosome behavior during microsporogenesis that can account for the wide differences in fertility found among the parental varieties, the hybrid, or inbred populations. If the presence of univalents and multivalents at metaphase I is the major cause of reduced fertility, the high-fertility class and the hybrid populations should have the lowest frequency of these configurations. The fact that the opposite condition occurs indicates that factors other than chromosome pairing at metaphase I must be more important in con-

TABLE III. Chromosome associations at metaphase I

Variety or Seedling	P.M.C. analyzed	Univalents Mean	Univalents Range	Bivalents Mean	Bivalents Range	Trivalents Mean	Trivalents Range	Quadrivalents Mean	Quadrivalents Range	Multivalents Mean	Multivalents Range	Meiotic chromosome count Mean	Meiotic chromosome count Range	Mitotic count
Parental Vines														
Folle blanche $2n$	11	0.2	0-2	16.5	11-19	–	–	1.3	0-4			38	38	38
Folle blanche $4n$	13	2.2	1-4	19.5	10-27	1.9	1-5	7.1	2-10			75.3	75-78	76
Cornichon $2n$	5	0.8	0-2	18.0	17-19	0.2	0-1	0.2	0-1			38	38	38
Cornichon $4n$	49	1.2	1-4	22.7	14-32	0.8	1-3	6.9	3-10			76.1	72-78	76
Zinfandel $4n$	15	3.3	2-8	23.1	15-28	1.1	1-2	5.9	3-11			76.8	73-80	76
Vines of High Fertility														
J 19-6	10	2.2	1-4	18.3	12-26	2.2	1-3	7.5	4-10	0.1	0-1	75.6	72-78	76
J 19-5 $4n \times 4n$	33	1.7	1-5	19.8	10-32	0.6	1-2	8.5	1-14	0.15	0-1	75.5	71-80	76
J 19-7	25	2.7	1-8	17.9	12-24	1.8	1-5	7.2	3-13	0.5	0-1	76.2	74-80	76
J 17-89 $4n$ Selfed	14	1.8	1-3	25.8	17-33	0.5	0-1	5.4	2-10	–	–	76.6	74-81	76
J 17-86	14	4.0	1-8	14.9	7-22	0.4	1-2	9.4	6-13	0.4	0-1	75.3	71-78	76
Mean		2.5		19.3		1.5		7.6		0.3		75.8		
Vines of Low Fertility*														
J 19-8 $4n \times 4n$	28	2.1	1-6	20.6	10-34	1.7	1-3	7.0	2-12	0.2	0-2	76.5	74-82	79
J 19-3	32	1.3	1-4	16.0	6-26	0.7	1-2	9.2	3-14	0.4	0-3	75.7	72-80	76
J 17-90 $4n$ Selfed	22	2.6	0-5	22.8	12-31	0.9	1-4	6.0	2-10	–	–	75.3	71-78	76
J 17-83	13	3.5	2-8	20.1	14-28	0.2	0-1	7.9	5-10	0.05	0-1	76.2	74-80	76
J 19-11 $4n \times 4n$	32	3.1	1-7	21.5	17-30	1.8	1-4	6.4	3-9	0.1	0-1	76.3	74-78	76
Mean		2.5		22.2		1.1		7.3		0.2		76.0		
Mean of $4n \times 4n$		2.2		19.0		1.5		7.6		0.2		76.0		
Mean of $4n$ Selfed		3.0		20.9		0.5		7.2		0.1		75.9		

*J 19-8 of medium fertility

trolling self-fertility. At later stages of microsporogenesis the greatest degree of irregularity is found in vines of high self-fertility, again casting doubt on chromosome behavior as an important factor determining gametic fertility.

According to Kostoff and Kendall[10] and Darlington[5] chromosome pairing with multivalent formation at metaphase I produces unequal distribution of chromosomes at later stages. This may account for much of the reduced fertility in their work. Results with *Vitis* indicate that the formation of univalents as well as multivalents at metaphase I and their subsequent distribution reduces fertility but that this behavior is not the most important factor governing gametic fertility.

These studies of self-fertility in tetraploid *Vitis* parallel in part those of Randolph[18] and Fisher[7] with autotetraploid maize. The inbred population had a lower mean fertility than did the hybrid population. Differences in fertility could be due in part to cumulative genetic factors and in part to frequency and distribution of single chromsomes or groups. Genetic factors apparently play an important part, since the hybrid vines exhibit a higher self-fertility than do the inbred vines.

Myers and Hill[12] demonstrated in *Dactylis* a significantly greater total number of univalents, lagging chromsomes, and micronuclei in their inbred plants (10 percent univalents) than in their parental plants (five percent univalents). Such differences did not exist in *Vitis* between inbreds (3.0 percent), hybrids (2.2 percent), and parental vines (1.2-3.3 percent).

In the absence of a better technique, pollen-germination tests using the hanging drop or agar plate are useful for measuring pollen viability, although results are not always consistent. The germination percentages shown in Table I are low when compared to tests run on similar pollens by Olmo[15] and Randall[17] using the same method. Results presented here were based on pollens that were a few weeks old, after field pollinations had been completed. Pollens of both diploid and tetraploid vines were treated alike.

The data obtained fail to indicate a close relationship among the behavior of aberrant chromosomes during microsporogenesis, the germination of pollen *in vitro* and the fertility of pollen. This is similar to the findings of Grun[8] with alfalfa and of Batra[1] with muskmelons. In *Vitis*, during microsporogenesis, vines in the high ovule-fertility class show a higher degree of chromosome irregularity than vines in the low ovule-fertility class. The high-fertility class should have a lower pollen-germination and a lower transmission of fertility through the pollen if chromosome behavior is the important factor in fertility. Since this condition does not occur factors other than chromosome behavior must be more important in controlling fertility.

Differences in the pollen-fertility between the classes of high and low self or ovule-fertility could not be demonstrated, possibly be-

TABLE IV. Unoriented chromosomes of meiosis

Stages of meiosis	Variety or selection	Total P.M.C.	Regular separation normal percent	Irregular separation — Univalents percent	Irregular separation — Groups percent	Irregular separation — Groups and univalents percent
		Parental Varieties				
AI, TI	Folle blanche 4*n*	99	67.7	31.3	0.0	0.0 (B)*
	Cornichon 4*n*	13	76.9	23.1	0.0	0.0
	Zinfandel 4*n*	55	30.9	16.4	27.3	20.0
MII	Folle blanche 4*n*	14	64.3	35.7	0.0	0.0
	Cornichon 4*n*	64	78.1	21.9	0.0	0.0
	Zinfandel 4*n*	21	23.8	23.8	33.3	19.1
AII, TII	Folle blanche 4*n*	24	75.0	25.0	0.0	0.0
	Cornichon 4*n*	19	68.4	31.6	0.0	0.0
	Zinfandel 4*n*	28	21.1	21.1	35.7	21.1
		High Fertility Class (5 vines)				
AI, TI		135	66.7	5.9	2.2	25.2 (2B)*
MII		489	26.2	22.3	25.1	26.4
AII, TII		123	29.3	30.1	26.0	14.6
		Low Fertility Class (5 vines)				
AI, TI		203	63.2	11.9	0.5	24.4 (4B)*
MII		453	60.3	23.0	9.7	7.0
AII, TII		140	61.4	12.1	22.9	3.6 (1B)*

*B = Bridge

cause of an inability to control the number of pollen grains applied to a stigma at pollination. Randall[17] has stated that more than 3000 pollen grains are applied to a stigma in a single hand-pollination. Any differences in self-fertility would be masked by the over-abundance of normal grains applied under such conditions. Demonstrating such differences would require a different pollinating technique so that the number of pollen grains applied be limited.

The lack of correlation between ovule-fertility and pollen fertility might be explained by a differential behavior between chromosomes in megasporogenesis and those in microsporogenesis. To account for the large differences in ovule fertility between different plants, however, variations in chromosome pairing and distribution during megasporogenesis would have to be large indeed—so large, in fact, that such variations would be better reflected in microsporogenesis. A more logical explanation would favor genetic differences that alter gametic fertility rather than the mechanics of chromosome pairing and distribution.

Recent reviews indicate that with most autotetraploid plants the metaphase I of microsporogenesis show a high frequency of quadrivalents and the presence of other multivalents. Later stages of meiosis indicate considerable irregularity, mostly lagging univalents and multivalents. The lagging chromosomes generally form micronuclei which are excluded or lead to an unbalanced condition in the microcytes. Fertility is reduced, reported Batra[1], Das[6], Grun[8], and Müntzing[11]. Such meiotic irregularities as occur in anaphasic separations would be expected to correlate with the numbers of microspores aborted. However, this does not appear to be true in muskmelons or alfalfa[1, 8].

It has been possible to improve the fertility of autotetraploids of several crop plants through several generations of crosses and selection, Bremer and Bremer-Reinders[3], Nilsson[13]. These workers found that the fertility of the autotetraploid segregates surpassed that of the autotetraploid parents but did not reach the level of fertility of the diploid plants. In *Vitis* it has been possible by hybridizing and selection in the autotetraploid to exceed the self-fertility of the autotetraploid parents as well as their corresponding diploid parents. Reduction in fertility that might be attributed to chromosome pairing and distribution is hence of relatively little importance in the breeding of new grape varieties at the tetraploid level.

The work of Randall[17] as well as the present study clearly indicates that in *Vitis* when the autotetraploid is used as the male parent and the diploid as the female parent the number of floating seeds is very great. There is little difference between the high and low classes of self-fertility in this respect, even though differences exist in the percent ovule set. This may indicate the possibility of a physiological disturbance involving the embryo, endosperm, and nucellus. Brink and Cooper[4] working with tobacco, and Sansome, Satina, and Blakeslee[19] working with *Datura* have shown that this disturbance may be caused by an unbalance between endosperm and nucellus or among embryo, endosperm, and nucellus. The disturbance seems to arise in the differences between the endosperm and nucellus, not involving the embryo. With respect to chromosme numbers there is a *3n-5n-4n* relationship when the tetraploid is the female parent, and a *3n-4n-2n* relationship when the tetraploid is the paternal parent, assuming that diploid gametes are produced by the tetraploid parent.

Summary

The self-fertility of autotetraploid grape varieties is about equal to the self-fertility of the corresponding diploid variety, but varies with the variety itself. Self-fertility varies from year to year, but nearly the same fertility ratio between the diploid and the corresponding tetraploid is maintained.

By selection it is possible to obtain F_1 hybrids from two tetraploid varieties that show a higher or lower self-fertility than that of the parental tetraploid or diploid varieties from which the hybrids were derived. The same is true of inbred seedlings from a tetraploid variety. Generally the hybrid seedlings show a higher self-fertility than the inbred seedlings.

Pollen from diploid vines show a uniformly higher germination than does pollen from the derived tetraploid vines. Only slight differences occurred in the germination percentages of tetraploid pollen, regardless of whether the variety showed high or low ovule-fertility. No close relationship between pollen-fertility and ovule or self-fertility can be established for individual vines by the methods used. There is a close correlation between self-fertility and ovule-fertility.

Using the tetraploid as the pollen parent gives a higher percentage of defective seeds than when using it as the seed parent.

At metaphase I all three autotetraploid varieties show approximately the same mean number of quadrivalents (5.0-7.1). The same holds true of the means (7.3). The mean number of univalents and other multivalents varies with the variety and may be partly related to the self-fertility. There is, however, not enough difference in the mean numbers of univalents and multivalents between vines of high and low self-fertility to account for the wide differences in self-fertility that do exist.

Fertility in autotetraploid *Vitis* is controlled in part by the behavior of univalents and multivalents during stages of meiosis, but other factors, such as the segregation of genes for fertility, seem more important.

Literature Cited

1. Batra, S. Induced tetraploidy in muskmelons. *Jour. Hered.* 43:141-148. 1952.

2. Bioletti, F. T. The seedless raisin grapes. *Cal. Agr. Exp. Sta. Bul. 298*:73-86. 1918.
3. Bremer, G. and D. E. Bremer-Reinders. Breeding of tetraploid rye in the Netherlands. I. Methods and cytological investigations. *Euphytica 3*:49-63. 1954.
4. Brink, R. A. and D. C. Cooper. Somatoplastic sterility. *Genetics 26*:487-504. 1941.
5. Darlington, C. D. Recent Advances in Cytology. Second ed. P. Blakiston's Son & Co., Inc. 671 pp. 1937.
6. Das, B. C. Cytological and embryological basis for sterility in autotetraploid sweetclover, *Melilotus alba* (Sesr.). *Iowa Sta. Coll. Jour. Sci. 27*:537-551. 1953.
7. Fisher, H. E. Causes of sterility in autotetraploid maise. *Genetics 26*:151 abs. 1941.
8. Grun, P. Variations in the meiosis of alfalfa. *Amer. Jour. Bot.* 38:475-482. 1951.
9. Kostoff, D. Evolutionary significance of chromosome length and chromosome number in plants. *Biodyamica* 51:1-14. 1939.
10. ———— and J. Kendall. Studies on polyploid plants. III. Cytogenetics of tetraploid tomatoes. *Gartenbauwiss* 9:20-44. 1934.
11. Müntzing, A. Cytogenetic properties and practical value of tetraploid rye. *Hereditas 37*:17-84. 1951.
12. Myers, W. M. and H. D. Hill. Meiotic irregularity in *Dactylis*. *Genetics 28*:383-397. 1943.
13. Nilsson, E. Tetraploid tomatoes. *Hereditas 36*:181-204. 1950.
14. Olmo, H. P. Empty-seededness in *Vitis vinifera* L. *Proc. Amer. Soc. Hort. Sci. 32*: 376-380. 1932.
15. ————. Storage of grape pollen. *Proc. Amer. Soc. Hort. Sci.* 41:219-224. 1942.
16. ————. Breeding tetraploid grapes. *Proc. Amer. Soc. Hort. Sci.* 59:285-290. 1952.
17. Randall, T. E. Triploidy in the European grape *Vitis vinifera*. *Ph.D. thesis*, unpublished, 47 pp. University of California, Berkeley, 1940.
18. Randolph, L. F. The influence of heterozygosis on fertility and vigor in autotetraploid maize. *Genetics 27*:163 abs. 1942.
19. Sansome, F. W., S. Satina and A. F. Blakeslee. Disintegration of ovules in tetraploid-diploid and in incompatible species crosses in Datura. *Bul. Torrey Bot. Club 69*: 405-420. 1942.

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AMINO ACID TURNOVER IN BRAIN COMPARED WITH TURNOVER IN OTHER TISSUES

Joseph W. Still*

AN EXPERIMENT has been conducted to compare the rate of amino acid turnover in brain tissue of mice with that of other organs and tissues. The technique used differs somewhat from the usual approach to the problem of protein turnover. Instead of employing the classical Schoenheimer technique of introducing tagged amino acids into fully formed animals, C_{14} labeled amino acids were built into mice by feeding these substances to the dam during the gestation and lactation period. Then the "rundown" in certain tissues was followed by killing the mice at various intervals after C_{14} feeding had stopped. A similar approach to the problem of hydrogen turnover has recently been reported by Thompson and Ballou[6]. They produced some rats whose dams were fed water containing tritium oxide during pregnancy and lactation. After weaning, the young rats were continued on the tritium labeled water until they were six months of age. The dams and the young were killed at various intervals after the tritium administration had stopped and the individual tissues ana-

*George Washington University, Washington, D. C. The generosity of Dr. Richard B. Roberts in providing the C_{14} labeled glycine and serine used in this experiment, the advice of Dr. Roberts and Dr. Dean Cowie of the Carnegie Institute of Terrestrial Magnetism, the low-level radioactivity assay by Dr. Ariel G. Schrodt and the assistance of Mrs. Mary McLaughlin of Walter Reed Army Institute of Research are gratefully acknowledged.

lyzed for tritium content. Although the metabolic pathways of tritium differ greatly from those of C_{14} labeled glycine and serine — the substances used in the present study — it is interesting that the results here reported, like those of Thompson and Ballou[7], indicate that there are great differences in the degree of dynamism of the constituents of different body tissues.

The hypothesis which suggested the experiment reported here was that since mitosis with cell renewal does not occur in adult nervous tissue, there may be relative (perhaps absolute) stability in the nuclear amino acids of nerve cells as compared with cytoplasmic amino acids of nerve cells and as compared with all amino acids in cells which do turn over during the life of the organism. The results obtained support a part of this hypothesis.

Material and Methods

A pregnant female mouse was placed on C_{14} labeled glycine and serine, dissolved in her drinking water, six days before her litter was born and continued for 12 days after the birth of the litter. In this period she drank a total of 250 cc. of water containing 100 microcuries of radioactivity. The water containing the radioactive amino acids was then replaced with ordinary tap water for the remainder of the experiment. Ordinary Purina rat chow was available at all times. The animals were killed at various intervals for analysis of their tissues.

The log of the experiment was as follows:

Day 1—C_{14} glycine and serine added to drinking water of pregnant mouse
Day 7—Litter born
Day 18—C_{14} amino acids stopped. Sibling (S_1) killed
Day 34—Dam and S_2 killed
Day 52—S_3 killed
Day 164—S_4 killed

As each animal was killed its tissues were separated for analysis as follows: (1) Brain; (2) Heart and Lungs; (3) Liver; (4) Kidney and Spleen; (5) Skeletal Muscle.

The wet tissue samples were weighed and then homogenized in glass homogenizers (manual type), adding sufficient distilled water to effect complete homogenization. The homogenate was then transferred to a Buchner porcelain funnel fitted with a retentive filter paper over the perforated surface. Suction was applied to the side arm of the funnel and the fluid extracted and discarded. The homogenized tissue on the filter paper was then dried and counted for 20 minutes or longer in a thin-walled, gas flow, low-level Geiger counter. Massive iron shielding and anti-coincidence counters were used to accomplish background reduction. The counting error of this instrument has been determined to be not greater than 20 percent for materials giving counts of 1 p.m., and less than 10 percent for those with counts of 25 p.m. when the samples are counted for 20 minutes or more.

Results

The results are shown in Table I. The data have been tested statistically by applying the t-test to the following comparisons:

Mean of	t value	P
$S_2S_3S_4$ Brain (34,39, 25-ave. 32.7) vs. $S_2S_3S_4$ Heart, Lung and Skeletal Muscle (10, 13, 7, 12, 9-ave. 10.2)	6.64	<1%
$S_2S_3S_4$ Brain (34, 39, 25-ave. 32.7) vs $S_2S_3S_4$ Liver, Kidney and Spleen (1, 2, 3, 0, 0-ave. 1.2)	10.26	<1%

The t-test values indicate that the probability of either of these differences being due to chance is much less than one percent.

Discussion

The dam's brain, heart and lungs contained very much less radioactivity than did the other tissues taken from her. These findings in the dam should be contrasted with S_1 which showed essentially uniform distribution of radioactivity in all tissues on the day the C_{14} amino acids were stopped. The findings in the dam's tissues should also be compared with S_2 killed the same day as the dam, as well as with S_3 and S_4 killed later, all of which, in contrast with the dam, showed high levels of radioactivity in their brains.

When S_2, S_3 and S_4 are compared with S_1 we observe retained radioactivity in nerve and muscle tissues while it is lost in other tissues. In order to determine the exact extent and location of nondynamic materials as indicated by this result and by the results of the experiment of Thompson and Ballou[7], it will be necessary to carry experiments of this general design for longer time periods and to use very precise tissue counting techniques and to make detailed chemical analyses of tissues produced in this way.

The hypothesis which has been partially tested by the experiment reported is of interest in relation to an aging theory presented by Still[5] which asked "What changes in cell physiology could account for the increased transmission time (with age) of nerve impulses?" This question and the theory from which it derived was inspired in part by the experiment of Sonneborn[4] in which he divided flat worms in half and found that the posterior-halves which regenerated a new head lived longer than did the anterior-halves which regenerated only a new tail. The writer has interpreted Sonneborn's results as being due to the fact that the worms with new heads had received new (youthful) vegetative (homeostatic) control centers whereas the animals with new tails still had their (old) original control centers. Hence the longevity of the latter worms was essentially unchanged by the procedure while the posterior halves growing new heads were thus rejuvenated.

The findings here reported add some confirmation to current understanding of "cell turnover" in different tissues. Current beliefs are based mainly on the study of mitotic indices of different tissues. Leblond and Walker[2] recently reviewed the literature dealing with this subject. Their review supported the general belief that higher animals develop their quota of nerve, (sense organ and adrenal medulla) and muscle cells early in postnatal life, and adults give no evidence of ability to replace lost cells in these tissues. "No mitoses at all (are seen) in (these tissues) of the adult rat," whereas most other tissues give mitotic evidence that they are constantly replacing cells at varying rates. The results reported here as well as those of Thompson and Ballou are consonant with Folin's concept of endogenous and exogenous protein metabolism. Folin's original concept has recently been reassessed in the light of present chemical knowledge by Mitchell[8].

The question of absolute vs. relative stability of the stable brain material will be of critical interest in relation to the "reverberating circuit" theory of memory which has been proposed by Gerard and Ransmeier[1], and in relation to the recent finding of absolute chemical stability in the chromosomal DNA of dividing vegetable (bean) cells. The latter finding was ported by Taylor, Woods and Hughes[6]. The implications of findings such as these will also be of interest in relation to problems of fallout.

Summary and Conclusion

An experiment is reported which demonstrates that when C_{14} was incorporated into the tissues of young mice by feeding C_{14} glycine and serine to their dam during the last six days of their intrauterine and first 12 days of their post-natal life. Radioactivity persisted for nearly five months in brain and muscle tissues whereas it disappeared completely from kidney, liver and spleen during this period.

The implications of this finding are briefly discussed.

Literature Cited

1. Gerard, R. What is memory? *Scient. Amer.* 189: 118. 1953.

2. Leblond, C. P. and B. E. Walker. Renewal of cell populations. *Physiol. Rev.* 36: 255. 1956.

3. Mitchell, H. H. The validity of Folin's concept of dichotomy in protein metabolism, *Jour. Nutrition* 55: 193. 1957

4. Sonneborn, Tracy. Genetic studies on *Stenostomun incaudatum*. *Jour. Exp. Zool.* 57: 57. 1930.

5. Still, Joseph W. Are organismal aging and aging death necessarily the result of death of vital cells in the organism? *Med. Ann. of the District of Columbia* 25: 199. 1956.

6. Taylor, J. H., P. S. Woods and W. L. Hughes. The organization and duplication of chromosomes as revealed by autoradiographic studies using tritium-labeled thymidine. *Nat. Acad. Sci.* 43: 122. 1957.

7. Thompson, R. C. and J. E. Ballou. The predominantly non-dynamic state of body constituents in the rat. *Jour. Biol. Chem.* 223: 795. 1956.

TABLE I. Counts per min. per gram of tissue (wet basis)

Animal	Dam	S_1	S_2	S_3	S_4
Day Killed	34	18	34	52	164
Brain	1	22	34	39	25
Heart and lungs	0	30	*	10	13
Liver	13	17	1	2	0
Kidney and spleen	15	33	*	3	0
Skeletal muscle	16	17	7	12	9

*These samples were lost through technical error.

MODIFIERS OF THE PLEIOTROPIC EFFECTS OF THE SHORT EAR GENE IN THE MOUSE

MARGARET C. GREEN*

THE phenotypes determined by mutant genes with major effects are often more variable than the normal phenotype. To the extent that this variability is genetic, it is said to be caused by "modifying genes." The relationship between major genes and their modifiers has been studied by, among others, Dunn and Charles who published a long series of papers on the relationship between the gene for piebald spotting and its modifiers in the mouse (see Grüneberg[11], p. 550 for references) and by Haskell[12] who worked with the polygenes affecting the manifestation of scute in *Drosophila*.

In the case of major genes with pleiotropic effects the question arises as to whether modifiers act alike on all effects of the gene or whether different modifiers act on different effects. Several investigations have shown that the pleiotropic effects of genes can vary independently on different genetic backgrounds, indicating that different modifying genes act on the various affected parts. These studies have mostly been concerned with apparently unrelated effects of pleiotropic genes, for example, limb and urogenital abnormalities as effects of luxate in the mouse[1], and tail and urogenital abnormalities as effects of Danforth's short tail, *Sd* in the mouse[2]. The present study deals with the modifiers of the short ear gene (*se*) which has multiple effects on a single system, the skeleton. The question is whether any of these modifiers, like the major gene, affect the system as a whole or whether there are different sets of modifiers acting on different parts of the system.

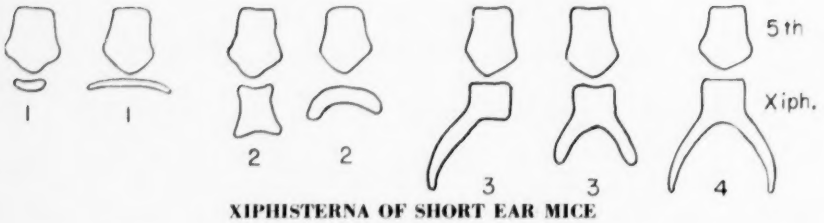
In *sese* mice the degree of development of the parts affected may be quite variable and this variation must be under both genetic and environmental control since it occurs both between and within inbred strains. Two inbred strains carrying *se* were available which were at opposite extremes for degree of development of most of the skeletal characters affected by *se*. These two strains were crossed and F_2 and backcross generations were produced. Correlation coefficients between the skeletal characters in segregating and non-segragating generations were determined and compared. It was assumed with some reservations that any tendency for higher correlations in segregating generations would indicate common action of the modifiers on the characters being correlated.

The results demonstrate little measurable increase in correlation in the segregating generations and support the conclusion that the modifiers do not act on the system as a whole.

Strains and Methods

The parental strains used were P/JaxGn (genotype *aa bb pp dse/dse*) which has minimum development of most of the skeletal characters affected by *se*, and NB/Gn (genotype *aa bb* $c^{ch}p/c^{ch}p$ *dse/dse*) which has maximum development of most of these characters. The P/JaxGn strain (referred to as P) was descended from a single pair in the 13th generation of brother-sister inbreeding obtained from the Jackson Memorial

*The data for this study were gathered at the Department of Zoology and Entomology, The Ohio State University, Columbus 10, Ohio. The analysis of the data was performed at the Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine, the present address of the author, and was partly supported by research grant NSF G-3033 from the National Science Foundation.



XIPHISTERNA OF SHORT EAR MICE

Figure 2

Types of xiphisternum and grades assigned to each. See text for details.

Laboratory, Bar Harbor, Maine in 1939. The NB/Gn strain (referred to as N) originated at Brown University[4,6], and has since been maintained at The Ohio State University. All P strain mice used were in the 39th to 45th generations of brother-sister inbreeding with the most recent common ancestors in the 30th generation. All N strain mice were in the 34th to 42nd generations of inbreeding with the most recent common ancestors in the 33rd generation. The parental strains were mated reciprocally. The F_2 was produced by sib matings of both reciprocal F_1's, and the backcrosses (B_P and B_N) were produced by matings of females of both reciprocal F_1's to pure strain males.

Mice for which skeletal data were to be obtained were killed at about 30 days of age and prepared for observation by a rapid alizarin staining method[8]. The following characters were recorded.

Number of pairs of ribs

Mice can have 12, 13, or 14 pairs of ribs. The short ear gene reduces the size of the last ribs[5,17]. The amount of reduction varies with the genetic background but in general it is a little less than one full pair of ribs. In this experiment the mice had either 12 or 13 pairs with the 13th pair occurring in all degrees of development. The 13th rib could be classified from 0 to 4 on each side, the maximum possible grade being 8 for complete development on both sides.

Number of sternebrae anterior to the xiphisternum

The normal number of sternebrae anterior to the xiphisternum in mice is five. There may, however, be an extra element between the fifth and the xiphisternum, or the fourth and fifth may be fused. The short ear gene causes a lower frequency of the extra element or an increase in the frequency of fusion[7]. In this experiment the extra element was never present but fusion often occurred. Thus mice could have either four or five elements anterior to the xiphisternum.

Xiphisternum

The xiphisternum is normally a long rectangular bone but in *sese* mice it is typically reduced in size or abnormal in shape[6]. In the N strain it is usually large and bifurcated and in the P strain it is usually small and occasionally absent. A grading scheme of five categories was devised. This is shown in Figure 2 with the 0 category representing complete absence of the xiphisternum not shown. Not all variations are represented in this scheme and it was necessary to classify the unusual variants according to the grade they most nearly resembled. Variation in both size and shape occurred and the grading scheme was an attempt to cover both aspects. Difficulties in classification were largely a result of the imperfect association between the two kinds of variation.

Anterior tubercle

The sixth cervical vertebra has a pair of ventral processes, the anterior or ventral tubercles (called inferior lamellae in a previous publication[7]). These may be reduced in size or absent on one or

THYROID CARTILAGES OF SHORT EAR MICE

Figure 3

Types of thyroid cartilage and grades assigned to each. See text for details.

both sides. If grades 0, 1, and 2 are assigned to absent, partial, and completely developed processes respectively, grades 0 to 4 are possible for both sides. Variation in this character occurs irrespective of *se*[10, 15, 16]. It is also affected by *se*, however, being absent with greater frequency in *sese* mice than in normal mice of otherwise comparable genotype[7, 13].

Ulnar sesamoid

This is a small bone in the wrist articulating with the pisiform bone[7,9]. In *sese* mice it is always either very much reduced in size or absent. Grades 0 to 2 were possible in this experiment depending on whether it was absent, present on one side, or present on both sides.

Peroneal process

This is a lateral extension of the head of the fibula. It is reduced in size or absent in *sese* mice[7]. Grades 0 to 2 were assigned depending on whether it was absent, present on one side, or present on both sides.

Medial fabella

This is a sesamoid bone on the medial side of the knee (called medial sesamoid in a previous publication[7]). It is nearly always reduced in size or absent in *sese* mice[7]. It was possible to recognize three size grades, 0, 1, and 2 for absent, very small, or medium sized (full development did not occur). This makes grades of 0 to 4 possible for both sides taken together.

Thyroid cartilage

The cartilages of the larynx are less well developed in *sese* than in normal mice[7]. This difference is best seen in preparations stained for cartilage. However, in mice 30 days old the cartilage has calcified sufficiently so that differences in the thyroid cartilage can easily be recognized after alizarin staining. This method was used in the present experiment. Figure 3 shows in ventral view the range of variation that occurred and the grades used in classification.

Results

Table 1 gives the distribution of each character in the parental and cross bred generations with the reciprocal F_1's listed separately. The numbers are not the same for all characters in each generation because of an occasional failure to classify for a character. The grades have been combined into two or three categories in a way so as to give minimum overlapping of the parental strains. The relative positions of the generations can be judged by following, say, the proportion in the highest grade or combination of grades. In the F_1 there are highly significant differences between reciprocals for ribs, sternebrae, peroneal process, and thyroid cartilage and a significant difference for anterior tubercle. In three cases (ribs, sternebrae, peroneal process) the F_1's tend to resemble the female parent, and in the other two cases (anterior tubercle, thyroid cartilage) they tend to resemble the male parent. The reciprocal differences are probably due to an effect of the maternal uterine environment although proof of this is lacking.

For all the characters except sternebrae there is a striking difference between the parents. The cross bred generations are more or less as expected if the differences between the parents are heritable, but the genetic differences between the generations are partly obscured by maternal effects in the five cases mentioned above. To determine more exactly whether the cross bred generations occupy the expected positions relative to the parent strains and to each other it would be necessary to correct for the maternal effect. This could be done by taking the difference between the reciprocal F_1's as a measure of the difference in maternal effect between the parent strains, and the difference between the actual F_2 and the expected F_2 (half way between the F_1 and the midparent) as a measure of the maternal effect of the F_1 females. However, the grading

scheme used to classify the characters was not an appropriate scale for determining the means of the generations and the choice of a more appropriate scale presented difficulties. For this reason and because it was not the main purpose of this study to investigate in detail the inheritance of each character, it did not seem worthwhile to pursue the matter further, even though correction for maternal effect would undoubtedly give a better fit of the cross bred generations to expectation.

The difference between the parent strains in number of sternebrae is not large, and, judging from the difference between the reciprocal F_1's, might be due entirely to maternal effects. If the difference between the parents is not directly gene controlled but only indirectly controlled through the genotype of the mother one would expect the F_2 and backcrosses to be alike since they were all produced by F_1 females. However the Bp has a significantly higher proportion with five sternebrae than the other two generations (F_2, B_P, $\chi^2 = 25.2$, df = 1, $P<.01$; B_P, B_N, $\chi^2 = 12.7$, df = 1, $P<.01$). Since the P strain was the parent with the smaller number of sternebrae, these results indicate that if there is a genetic difference between the parents affecting sternebrae directly it must be in the opposite direction from the phenotypic difference actually observed.

There were significant differences between the sexes for most of the characters in at least one generation although there was in most cases little consistency from generation to generation. Not all generations could be tested for some of the characters, notably ulnar sesamoid, because of lack of sufficient variation. Where more than one generation showed a significant effect of sex, the differences were always in the same direction for each character. Thus, males tend to have more pairs of ribs than females, more sternebrae, smaller xiphisterna, fewer anterior tubercles, and smaller peroneal processes and medial fabellae. There is no effect of sex on thyroid cartilage and no consistent effect on ulnar sesamoid.

Genetic Associations

Association with C and c^{ch}

Only one major gene difference was involved in the cross, i.e. *C* and c^{ch}, the alleles for full color and chinchilla at the albino locus in linkage group I. Segregation could be observed in F_2 and B_N and association of the characters with this segregation could be tested

TABLE I. Distribution of eight characters in the parental and cross bred generations of a cross between two inbred strains. Figures are percentage of total number

	Grade	P		N		F_1 ♀P/♂N		F_1 ♀N/♂P		F_2		B_P		B_N	
		♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
Ribs	0	88	91	–	–	–	–	–	–	3	4	22	32	–	–
	1-7	12	9	–	2	41	44	24	16	38	47	73	57	17	17
	8			100	98	59	56	76	84	59	49	4	11	83	83
	No.	59	55	59	56	54	46	58	57	328	299	187	196	221	190
Sternebrae	4	29	49	14	12	41	50	9	29	40	57	25	40	37	54
	5	71	51	86	88	59	50	91	71	60	43	75	60	63	46
	No.	59	55	59	56	54	46	58	56	326	296	187	195	221	190
Xiphisternum	0-2	100	98	2	–	22	24	24	18	62	51	79	73	30	32
	3-4		2	98	100	78	76	76	82	38	49	21	27	70	68
	No.	59	55	59	56	54	46	58	56	326	295	187	195	221	190
Anterior tubercle	0-1	100	100	3	–	44	26	50	35	62	46	91	87	16	14
	2-4			97	100	56	74	50	65	38	54	9	13	84	86
	No.	59	55	59	56	54	46	58	57	328	299	187	196	221	190
Ulnar sesamoid	0	100	100	–	–	2	2	–	–	21	21	74	77	1	
	1-2			100	100	98	98	100	100	79	79	26	23	99	100
	No.	59	55	59	56	54	46	58	57	326	296	187	196	221	189
Peroneal process	0	93	91	42	30	78	41	64	33	51	30	70	40	37	28
	1-2	7	9	58	70	22	59	36	67	49	70	30	60	63	72
	No.	59	54	59	56	54	46	58	57	327	299	186	196	221	189
Medial fabella	0-2	90	96	7	–	6	4	12	9	25	15	43	30	8	8
	3-4	10	4	93	100	94	96	88	91	75	85	57	70	92	92
	No.	59	55	59	56	54	46	57	57	327	299	186	196	221	188
Thyroid cartilage	0-1	100	89	22	9	11	9	19	28	32	34	67	62	24	21
	2		11	78	91	89	91	81	72	68	66	33	38	76	79
	No.	59	55	59	56	54	46	58	57	328	299	187	196	221	190

to determine if genes in this linkage group have an effect on any of the characters. The results of these tests are given in Table II.

In F_2 five of the characters (ribs, anterior tubercle, ulnar sesamoid, medial fabella, and thyroid cartilage) show an association with the albino locus, and three (sternebrae, xiphisternum, and medial fabella) do not. In all cases of association a lower grade of development of the character is associated with *C* which came into the cross from the parental strain with the lower grade (P), and a higher grade is associated with c^{ch} which came from the parental strain with the higher grade (N). This is what one would expect and supports the conclusion that the associations found are not accidental but are due to linkage with the *c* locus of a gene or genes affecting these characters. *C* and c^{ch} were also segregating in B_N but in this generation they were not significantly associated with any of the characters. This result can be explained by assuming dominance in the gene pair or pairs responsible for the associations observed in F_2 and linkage with the *c* locus in the repulsion phase. It seems very likely from the data given in Table I that there is some dominance of genes from the N strain since the distributions of the five characters in the F_1's lie either very near the distribution of N or between N and the midparent.

In a cross between BALB/c and C57BL in which three pairs of color genes (*C-c, A-a, B-b*) were segregating, Green[3] found an association of number of pairs of ribs with the *B-b* segregation but none with *C-c*. This in no way contradicts the conclusions from the present experiment, since it is quite possible that BALB/c and C57BL are alike at the locus affecting rib number which is segregating in linkage group I in this cross.

Association between characters

Tetrachoric correlation coefficients were calculated for association of each of the eight characters with all of the others in each generation except in the cases where there was not enough variation to allow it. In most cases the grades had to be combined in some way to give the two classes necessary for the calculation of tetrachoric r. This was always done so as to give the most nearly equal distribution of numbers in the two groups. The coefficients were calculated separately for the sexes and reciprocals in all cases where both of the characters were affected by these variables. The correlation coefficients were then averaged using the formula of Wright[18].

The parental and F_1 generations give a measure of the nongenetic correlation. Since the numbers were small in all three, the correlations were averaged for the three genera-

TABLE II. Relation of eight characters to segregation of *C* and c^{ch} in F_2 and B_N

		F_2				B_N			
	Grade	No.	%C—	χ^2	P	No.	%C—	χ^2	P
Ribs	0-5	125	84.8						
	6-7	162	81.5			71	56.3		
	8	340	69.1	16.4	< .01	340	51.8	0.5	.50-.30
Sternebrae	4	300	78.7			183	55.2		
	5	322	72.7	3.0	.10-.05	228	50.4	0.9	.50-.30
Xiphisternum	0-1	86	80.2			126	55.6		
	2	267	72.7						
	3	220	77.7			202	49.1		
	4	48	75.0	2.8	.50-.30	83	56.6	2.0	.50-.30
Anterior tubercle	0	287	81.9			62	56.5		
	1	53	77.4						
	2	157	70.1			108	50.9		
	3	130	66.9			42	45.2		
	4			14.1	< .01	199	53.8	1.5	.70-.50
Ulnar sesamoid	0	130	88.5			3	66.7		
	1	112	81.2						
	2	380	69.2	2.19	< .01	407	52.6	—	—
Peroneal process	0	255	72.2			134	49.2		
	1	101	81.2			46	52.2		
	2	270	76.7	3.4	.20-.10	230	54.8	1.0	.70-.50
Medial fabella	0-2	126	84.9			32	43.8		
	3	84	81.0			49	53.1		
	4	416	71.6	10.8	< .01	328	53.4	1.1	.70-.50
Thyroid cartilage	0-1	205	81.0			94	58.5		
	2	422	72.8	5.0	.05-.01	317	50.8	1.7	.20-.10

tions to give a more reliable measure. In the upper right half of Table III are given the nongenetic correlations, that is, the correlations averaged for P, N, and F_1, and in the lower left half the correlations for F_2, B_P and B_N minus the nongenetic correlations. It should be noted that these differences are not equivalent to genetic correlations but could be converted to them if the heritabilities of the eight characters were known. Heritabilities could not be calculated with any reasonable degree of precision from the present data and in any case the standard errors of the genetic correlations obtained from them would be unknown. The differences are therefore used directly. Any real increase in correlation in the F_2 and backcross generations should be due to genetic determiners common to the two characters or to separate determiners linked with each other.

It is possible to calculate standard errors for tetrachoric correlation coefficients using Pearson's tables[14], although their use is valid only if the assumptions underlying the method are fulfilled. There is some doubt as to whether this is so, but we can at least formally determine the significance, and regard as significant any differences which greatly exceed the significance level or for which there is other supporting evidence. The standard errors of the averages of the non-segregating generations were about 0.10 except in the case of correlations involving ulnar sesamoid. These correlations could only be computed in the F_1 and the standard errors are therefore much larger (about 0.18). The standard errors for the correlations in F_2 are about 0.07 and for the B_P and B_N about 0.09. Therefore, to be significant at the five percent level the differences between the tetrachoric r's must exceed 0.24 in the F_2 and 0.26 in the backcrosses, except for correlations involving ulnar sesamoid which must exceed 0.38 and 0.40 respectively. By this criterion only the five differences starred in Table III are significant and four of these are negative. Most of the other differences are either small or not consistent from generation to generation. While there is thus little evidence for genes with strong general effects on all the characters, there is evidence for the following more limited associations.

Ulnar sesamoid and medial fabella show a significant genetic association in F_2 and a high though not significant association in B_P. It will be recalled that both these characters show association with the *c* locus. It thus seems highly likely that there is at least one gene or a group of linked genes in the first linkage group affecting both characters.

With one exception all the associations involving peroneal process are negative and three of these, ribs, anterior tubercle, and

TABLE III. Average tetrachoric correlation coefficients between the eight characters for the nonsegregating generations in upper right. Tetrachoric correlation coefficients for the segregating generations minus those for the average of the nonsegregating generations in lower left. Ri = ribs, St = sternebrae, Xi = xiphisternum, At = anterior tubercle, Us = ulnar sesamoid, Pp = peroneal process, Mf = medial fabella, Tc = thyroid cartilage

		Ri	St	Xi	At	Us	Pp	Mf	Tc
Ri			.20*	-.03	.23*	-.06	.20*	-.03	.04
St	F_2	.10		.20*	.16	.00	.01	.00	-.04
	B_P	.22							
	B_N	.02							
Xi		-.06	.03		.07	.00	.20*	.03	.03
		.10	.06						
		.16	.11						
At		.05	-.06	-.26*		.00	-.03	-.01	.00
		-.08	.14	-.08					
		-.13	.03	-.11					
Us		.14	-.02	-.01	.08		.27	-.27	-.18
		.18	-.05	.06	-.03				
		—	—	—	—				
Pp		-.20	-.16	-.16	-.13	-.27		.48*	.13
		-.36*	-.09	-.12	-.30*	-.05			
		-.22	-.13	-.18	-.01	—			
Mf		.08	-.01	-.04	.04	.46*	-.20		-.01
		.03	-.04	.09	-.22	.37	-.07		
		-.05	.03	.05	.02	—	.10		
Tc		-.02	.06	.03	.02	.27	-.27*	.09	
		-.16	-.08	-.07	.24	.04	-.11	.03	
		-.16	.05	.08	.12	—	-.08	-.01	

*$P < .05$

thyroid cartilage, are significant. This may indicate that there is a gene lowering the expression of peroneal process linked to one or more increasing the expression of ribs, anterior tubercle, and thyroid cartilage, and possibly of the other characters also. Since the other characters do not all show positive correlations with each other it is likely that the gene affecting peroneal process has a stronger effect than the gene or genes affecting the other characters. This explanation is not, of course, the only one which could account for the observed negative correlations.

Anterior tubercle and xiphisternum show negative genetic association in F_2. This may be fortuitous, since the associations in the backcrosses, while still negative, are small.

The nongenetic correlations which are high enough to be formally significant are starred in Table III. The only one high enough to be surely significant is that between peroneal process and medial fabella. These are both bones of the knee and apparently are affected by some of the same environmental conditions.

Discussion

In the present experiment one would expect to find genetic intercorrelations between most of the characters if there are genetic factors which have a wide range of action over all or most of the characters. The results do not support the conclusion that there are such factors. Before the conclusion that there are no such factors can be reached, however, it would be well to examine some of the limitations inherent in the material and methods used.

1. The characters are not of a kind that can be measured on a continuous scale. They are to a certain extent all or none characters and must therefore be recorded as grades of discrete variables. Even in the case of the last rib, which could have been measured on a continuous scale, the distribution would have been far from normal in form. The use of the tetrachoric correlation coefficient implies the assumption that there is a continuous normal distribution underlying the discontinuous distribution of the variables. There is no way of testing the validity of this assumption, but even if it is true the proportion of individuals in each category is a very insensitive measure of the underlying scale.

2. The observations are partly subjective and therefore must contain a relatively large grading error.

3. Most of the characters have a large amount of nongenetic variation as can be seen in the F_1 distributions.

With such large sources of error, genetic sources of variation common to most of the characters could be easily obscured. That this may be the case is suggested by the fact that five of the characters showed association with the albino locus in F_2. There is no environmental variation or measurement error in the classification of the *c* locus. When, however, one looks for genetic association of these characters with each other (which they should show if they are affected by a gene or genes linked with *c*) one finds that of the ten expected associations, only one is significant (ulnar sesamoid and medial fabella in F_2), and one more is relatively high though not significant (anterior tubercle and thyroid cartilage in B_F). It is possible that some of the genes are on opposite sides of the *c* locus and far enough apart so as to recombine nearly independently. But the smallest number of associations to be expected under this scheme is four if there are three genes on one side and two on the other. It therefore seems likely that if there are true genetic associations between the characters in this experiment some of them will have escaped detection.

Even with these limitations in mind, however, it is hard to escape the conclusion that the modifiers of *se* present in this cross do not have general effects similar to those of *se*. The fact that most of the significant correlations which did occur were negative is additional evidence for this conclusion. The modifiers of *se* seem to be genes with local effects. Those affecting characters which can vary independently of the presence of *se*, namely ribs, sternebrae, anterior tubercle, medial fabella, and thyroid cartilage, probably are not dependent for expression on the presence of this gene and therefore should not strictly be thought of as modifiers. The others affecting xiphisternum, ulnar sesamoid, and peroneal process, appear to act as specific modifiers, but may very well have other effects which have not yet been detected. There seems to be no tendency for the "specific modifiers" to be more general in their action than the "non-specific modifiers."

Associations observed in the segregating generations following a cross may be due either to the pleiotropic action of segregating genes or to the association of linked genes. Without further testing there is no way of distinguishing between these two possibilities. Had all eight characters been closely associated it is unlikely that linkage could have been responsible, since it is unlikely that genes for all characters would be located on one chromosome. In the present case, however, where the associations are few and weak, linkage is at least as good an explanation as pleiotropy. The fact that some of the associations were negative slightly favors linkage as an explanation. A gene could act in opposite directions on two characters if it favored one over the other in competition for some limited material, but this seems a less likely explanation of the negative correlations than linkage of genes with opposite effects on the two characters. It should be pointed out here that the association of several of the characters with segregation at the *c* locus may be a result of pleiotropic action at this locus, although this seems a less likely explanation than linkage.

The question of linkage versus pleiotropy,

while not resolvable with the present data, could be solved by a selection experiment. The characters will respond to selection in opposite directions if their correlation is due to linkage, but not if it is due to pleiotropy.

Of the five significant genetic associations found, two were in B_F (peroneal process with ribs, and with anterior tubercle) and none were in B_S. Association in one backcross and not in the other would be expected to occur if there were dominance in at least one of the loci responsible for the correlation. As we have seen there was evidence from the F_1, and from the tests for association with the *c* locus, for dominance of alleles from the N strain affecting ribs and anterior tubercle. The evidence from the backcross correlations also supports this conclusion. The F_2 correlations in these two cases were high but not significant. This is to be expected since, where dominance exists, the F_2 is not as efficient for detecting genetic association as the backcross to the parent carrying the recessive.

Summary

The action of the genetic modifiers of the short ear gene (*se*) on eight skeletal characters affected by *se* were studied in a cross between two inbred strains homozygous for *se* but differing in grade of development of the eight characters. Tetrachoric correlation coefficients between all of the characters taken in pairs were determined for the parental strains, F_1, F_2, and the two backcrosses.

With few exceptions there was little increase in correlation in the segregating generations over the nonsegregating generations. This indicates that most of the modifiers do not have a general action comparable to that of *se* over all the characters but are local in their effects.

Five of the characters were associated with the segregation of *C* and c^{ch} in the F_2 but were not so associated in the backcross to the strain carrying c^{ch}. This indicates that there are one or more loci affecting five characters in linkage group I and that there is probably dominance between the alleles involved in this cross.

Literature Cited

1. Carter, T. C. The genetics of luxate mice. I. Morphological abnormalities of heterozygotes and homozygotes. *Jour. Genet.* 50: 277-299. 1951.

2. Dunn, L. C. and S. Gluecksohn-Schoenheimer. Dominance modification and physiological effects of genes. *Proc. Nat. Acad. Sci.* 31: 82-84. 1945.

3. Green, E. L. The genetics of a difference in skeletal type between two inbred strains of mice. *Genetics* 36: 391-409. 1951.

4. Green, E. L. and M. C. Green. The development of three manifestations of the short ear gene in the mouse. *Jour. Morph.* 70: 1-19. 1942.

5. ————. Effect of the short ear gene on number of ribs and presacral vertebrae in the house mouse. *Amer. Nat.* 80: 619-625. 1946.

6. Green, E. L. and C. W. McNutt. Bifurcated xiphisternum and its relationship with short ears in the house mouse. *Jour. Hered.* 32: 94-96. 1941.

7. Green, M. C. Further morphological effects of the short ear gene in the house mouse. *Jour. Morph.* 88: 1-22. 1951.

8. ————. A rapid method for clearing and staining specimens for the demonstration of bone. *Ohio Jour. Sci.* 52: 31-33. 1952.

9. Greene, E. C. Anatomy of the Rat. Amer. Philosophical Soc., Philadelphia. 1935.

10. Gruneberg, H. Genetical studies on the skeleton of the mouse. I. Minor variations of the vertebral column. *Jour. Genet.* 50:112-173. 1950.

11. ————. The Genetics of the Mouse. Martinus Nijhoff, The Hague. 1952.

12. Haskell, G. M. L. The polygenes affecting the manifestations of scute in *Drosophila melanogaster*. *Jour. Genet.* 45:269-276. 1943.

13. McNutt, W. Gene expression as influenced by genetic background. *Jour. Hered.* 45: 235-240. 1954.

14. Pearson, K. On the probable error of a coefficient of correlation as found from a fourfold table. *Biometrika* 9: 22-27. 1913.

15. Searle, A. G. Genetical studies on the skeleton of the mouse. IX. Causes of skeletal variation within pure lines. *Jour. Genet.* 54: 68-102. 1954.

16. ————. Genetical studies on the skeleton of the mouse. XI. The influence of diet on variation within pure lines. *Jour. Genet.* 52: 413-424. 1954.

17. Snell, G. D. The induction by x-rays of hereditary changes in mice. *Genetics* 20: 545-567. 1935.

18. Wright, S. An analysis of variability in number of digits in an inbred strain of guinea pigs. *Genetics* 19: 506-536. 1934.

KARYOTYPE ANALYSIS OF ORNITHOGALUM TEMPSKYANUM

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RECOGNITION of the value of karyotype analysis in studies of species relationship dates from the early investigations of Delaunay[3] and Lewitsky[5] on chromosome morphology in various plant genera including *Ornithogalum*. In the present study the karyotype of *O. tempskyanum* is described and compared to those of related species.

Of the 73 species of *Ornithogalum* recognized by Baker[1] the chromosome number has been determined for only 26[2] and studies of chromosome morphology have been limited to *O. exscapum* and *O. pyramidale* L. var *narbonense*[6], *O. thyrsoides, O. zeyheri, O. pyrenaicum, O. umbellatum, O. pyramidale, O. arcuatum, O. unifolium, O. concinnum, O. arabicum, O. divergens* and *O. narbonense*[8,9]. Similar observations on additional species are needed as a basis for more adequately evaluating the usefulness of karyotype analysis in studies of species relationship in this genus.

Specimens of *Ornithogalum tempskyanum* Freyn and Sint. were obtained from the botanical garden of the University of Pisa in March, 1954 along with various species of dwarf bearded iris, the seed having been received at Pisa from the Hortus Bergianus, Stockholm, Sweden. When the plant bloomed at Ithaca, N. Y. it was identified by Professor R. T. Clausen as *O. tempskyanum*, a species not known to have been grown previously in this country. It can be seen from the photograph taken while in bloom (Figure 4*A* and *B*) that there is close agreement with the original description of *O. tempskyanum* by Freyn[4]. No previous illustration of this species has been published according to the Index Londinensis and Index Kewensis to 1950.

The root-tip squash technique developed for iris karyotype analyses by Mitra[7] was found to be well suited to studies of chromosome morphology in this species of *Ornithogalum*.

The Pisa specimens of *O. tempskyanum* have nine pairs of chromosomes in the root tip cells (Figure 5*A* and *B*). These could be classified readily on the basis of length differences and the customary procedure of numbering the chromosomes in order from the longest to the shortest was followed. The shortest chromosome is about one-third the length of the longest chromosome and uniform length differences delimit the members of the genome.

Likewise, the position of the centromere differs in each of the chromosomes. Chromosome 1, the longest member of the set, has a median centromere and chromosome 2 has a submedian centromere. The four chromosomes of intermediate length, chromosomes 3 to 6, also have submedian centromeres but with shorter short arms than chromosome 2. The satellite chromosome, number 7, has a subterminal centromere and a satellite arm consisting of a conspicuous knob with a relatively long attachment filament. The two shortest chromosomes, 8 and 9, have essentially subterminal centromeres.

From an examination of Delaunay's[3] drawing of the chromosomes of *O. tempskyanum* it is apparent that in his material the chromosomes were similar to those of the Pisa plants except that the two shortest chromosomes in his figure appear to have median centromeres. In

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BLOOMS OF *O. TEMPSKYANUM*

Figure 4

A—A plant of *O. tempyskyanum* in bloom under greenhouse conditions, approximately one and a half times natural size. *B*—An individual bloom at about four times natural size.

Table I are given the lengths of the chromosomes of *O. tempskyanum* reported by Delaunay and those obtained in the present study.

Minor differences in the length of some of the chromosomes is apparent and the total length of the members of the set was somewhat greater in our material than in Delaunay's preparations. This is not unexpected since different methods of fixing the chromosomes were used and some fluids tend to shorten the chromsomes more than others.

Other species of *Ornithogalum* illustrated in the figures published by Martinoli[6] and Neves[8,9] have karyotypes characterized by obvious length differences

TABLE I. Lengths of the chromosomes of *O. tempskyanum* in microns

	I	II	III	IV	V	VI	S*	M_1	M_2	Total length
Delaunay's Data	13.0	9.5	8.0	7.0	5.0	4.0	5.5	2.5	2.5	57.0
	1	2	3	4	5	6	7	8	9	
Present Data	12.5	10.0	8.0	7.0	6.5	5.5	5.5	4.5	4.0	63.5

*This chromosome and chromosome 7 of our data are the satellite chromosomes; m_1 and m_2 denoted metacentric chromosomes in Delaunay's figure.

SOMATIC CHROMOSOMES OF *O. TEMPSKYANUM*

Figure 5

A—Photomicrograph of somatic chromosomes from a root-tip cell of *O. tempskyanum* (× 1,900). *B*—A camera lucida drawing of the same group of chromosomes.

among the chromosomes similar to the length differences found in *O. tempskyanum*. The karyotype of *O. exscapum* ($2n = 18$), illustrated by Martinoli, includes chromosomes morphologically very similar to those we have observed in *O. tempskyanum*. Furthermore, the morphology of the satellite chromosome is similar in both species; this chromosome in each of these species has a conspicuous satellite knob connected by a long thread.

From a comparison of the relative lengths of the chromosomes of these two species given in Table II, it can be seen that similar differences distinguish the members of each complement. Furthermore, the length decrements of the chromosomes from the longest to the shortest are essentially the same in both spe-

TABLE II. Comparative length differences in microns of the chromosomes of *O. exscapum* and *O. tempskyanum*

O. exscapum	A	B	C	D	E	F	I*	G	H
	18.5	16.4	14.7	10.6	10.0	9.1	9.3	7.0	6.6
O. tempskyanum	1	2	3	4	5	6	7	8	9
	12.5	10.0	8.0	7.0	6.5	5.5	5.5	4.5	4.0

*The sequence of this chromosome has been changed so that the relative position of the satellite chromosome is the same in each genome.

cies. The measurements indicate that the chromosomes of *O. exscapum* are somewhat longer than those of *O. tempskyanum*, but this apparent difference could be due to a more pronounced shortening of the chromosomes in one species than in the other during fixation.

Discussion

Determinations of chromosome number in *Ornithogalum* by various workers have established the occurrence of a dysploid series including the gametic numbers 3, 5, 6, 7, 8 and 9. It is at present uncertain whether the basic number in this genus is three or some higher number from which the numbers characteristic of various species have been derived by the addition or loss of chromosomal elements.

According to the concept of karotype evolution proposed by Lewitsky[5], genomes with symmetrical karyotypes having metacentric chromosomes of about equal length are considered to be less specialized than are those with acrocentric chromosomes of differing lengths.

It has been shown by Quintanilha and Cabral[10] that *O. virens* ($n = 3$) and *O. zeyheri* ($n = 6$) lack metacentric chromosomes, whereas species with higher numbers, which include the *O. tempskyanum* ($n = 9$) of this investigation and *O. exscapum* ($n = 9$) studied by Martinoli[6], possess metacentric chromosomes. This suggests the possibility that species of *Ornithogalum* with low chromosome numbers may have been derived from others with higher numbers. But additional cytotaxonomic studies are needed as a basis for conclusions about relationships in this genus.

The chromosomes of *Ornithogalum* are very favorable for studies of their morphology and numerical relationships, and furnish suitable material for the study of species affinities in this genus.

Summary

The nine chromosomes of the *O. tempskyanum* genome differ in length by essentially uniform increments, the shortest member of the set being only about one-third the length of the longest. The position of the centromere differs in each of the chromosomes. In No. 1, the longest chromosome, it is median, in No. 2 it is submedian and in the remaining seven chromosomes it shifts in a terminal direction with the two shortest chromosomes, 8 and 9, having essentially subterminal centromeres. The satellite chromosome, No. 7, has a conspicuous satellite attached by a long thread.

The karyotype of *O. tempskyanum* is similar to that of *O. exscapum* studied by Martinoli and, on the basis of the Lewitsky concept of karyotype differentiation, both are less highly specialized than are the karyotypes of species with lower chromosome numbers.

Literature Cited

1. Baker, J. G. Revision of the genera and species of Scilleae and Chlorogaleae. *Jour. Linnean Soc. Bot.* 13: 209-292. 1873.

2. Darlington, C. D. and A. P. Wylie. Chromosome Atlas of Flowering Plants. George Allen and Unwin. London. 1955.

3. Delaunay, L. N. Phylogenetische chromosomenverkürzung. *Zeitschr. Mikr. Anat.* 4: 338-364. 1926.

4. Freyn, J. Uber neue und bemerkenswerthe orientalische Pflanzenarten. *Bull. Herb. Boiss.* 4: 188. 1896.

5. Lewitsky, G. A. The karyotype in systematics. *Bull. Appl. Bot. Genet. and Plant Breed.* 27: 220-240. 1931.

6. Martinoli, G. Contributo alla caryologia del genere Ornithogalum. *Caryologia* 3: 156-164. 1950.

7. Mitra, Jyotirmay. Karyotype analysis of bearded iris. *Bot. Gaz.* 117: 265-293. 1956.

8. Neves, J. de B. Estudos cariológicos no género *Ornithogalum* L. *Bot. Soc. Brot.* 26: 1-192. 1952.

9. ————. Sobre o emparelhamento somático em *Ornithogalum zeyheri* Baker. *Bot. Soc. Brot.* 27: 203-216. 1953.

10. Quintanilha, A. and A. Cabral. A new species of Liliaceae with six somatic chromosomes. *S. Afr. Jour. Sci.* 43: 167-169. 1947.

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Travel Assistance Asked for Foreign Geneticists

A number of geneticists will be coming to the International Genetics Congress in Montreal in August, 1958. Undoubtedly, some of them can come before the Congress and some can stay after the Congress. Undoubtedly, also, some would like to visit laboratories in the United States, but to do so they will need dollars. The Travel Assistance Committee for the Congress is anxious to know wrich laboratories would like to help foreign geneticists by inviting one or more either to give a lecture or to come as a consultant on research. If you have any funds for lectures or for consultations which you could allocate to geneticists would you let the Committee know, as soon as possible, preferably before December 1st.

—Harriet B. Creighton
Wellesley College, Wellesley 81, Mass.

THE SEX OF PARTHENOGENETIC TURKEY EMBRYOS

H. K. Poole and M. W. Olsen*

THIS study was initiated to confirm histologically the sex of parthenogenetic embryos which developed in eggs laid by virgin Beltsville Small White turkey hens. Sixty-seven parthenogenetic embryos, ranging from 16 days of incubation to hatching, had been examined macroscopically and classified as males. This diagnosis was based on two gross characteristics of the gonads: (a) the long cylindrical shape typical of embryonic testes, and (b) the invariable occurrence of the right gonad which, though slightly smaller than the left, never resembled the atrophic right gonad typical of the female.

Swift[2, 3] and Willier[5] reported it possible to distinguish the sex of chick embryos as early as seven days of incubation, i.e. when the embryo had been incubated for approximately one-third of its normal total incubation time. Since the turkey embryos used in this study were incubated at least 16 days or approximately one-half of the total incubation period of 28 days, it was assumed that differentiation had proceeded far enough to allow definite sex characterization of the gonads.

Materials and Methods

The gonads were removed from embryos which had been fixed *in toto* in Bouin's fixative following 16 to 29 days of incubation. They were imbedded in paraffin, sectioned at ten microns, and stained with Heidenhain's Iron Hematoxylin. The sex of each embryo was determined following the criteria suggested by Swift[3] for the chick embryo.

In addition, the macroscopic and microscopic appearance of the parthenogenetic gonads was compared with that of normal turkey gonads of known sex at 12, 15, and 19 days of incubation.

Results and Discussion

All of the observed histological characteristics of the gonads examined indicated the sex of the parthenogenetic turkey embryo to be male. The "germinal epithelium," of cuboidal cell type, closely resembled its homolog in the normal embryonic turkey and chicken gonad. The sex cords or seminiferous tubules were well developed and were distributed throughout the gonad. Some of the gonads were from embryos of sufficient age that advanced lumen formation appeared in the seminiferous cords. Spermatogonia, Sertoli cells, and interstitial cells were located in the seminiferous tubules or sex cords. Only an occasional spermatogonium was observed in the process of cell division. In some of the gonads from older embryos the spermatogonia were observed to have a mitochondial crescent, located in the cytoplasm as in normal embryonic testicular tissue. In none of the gonads could a distinction be made between medullary and cortical regions as would be expected if the embryo were female.

These histological observations confirm completely the diagnosis of sex based on observations of the intact gonad.

Although no cytological evidence was observed to explain the occurrence of males exclusively, there are several possible hypothetical explanations.

In turkeys the female is heterogametic and therefore produces two types of gametes—one containing the single sex chromosome and the other containing no sex chromosomes. If chromosome doubling occurred in the haploid egg cell, the product of the former type would be a diploid cell with two sex chromosomes and therefore male while the latter would result in a cell without sex chromosomes. Further, if we assume the YY condition to be lethal as has been indicated in *Drosophila*[1], then only the male would be viable.

Yao and Olsen[6] attempted to determine whether the cells of parthenogenetic turkey embryos were haploid or diploid. They counted the chromosomes in cells of parthenogenetic extraembryonic membranes and found that the number ranged between 60 and 82. These findings correspond favorably with the diploid number of 76 male and 77 female reported by Werner[4]. The evidence suggests that a chromosome doubling occurred somewhere during parthenogenetic development involving nearly all if not all of the chromosomes in the haploid gametic complement. Yao and Olsen believed

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this doubling to occur as do the present authors.

Should this suggestion not be true, one is left with two alternatives: one being failure during oogenesis to expel the second polar body after anaphase II and the other reentrance of one of the polar bodies after completion of the second maturation division. On the first alternative, the results would be essentially the same as if chromosome doubling had occurred in the haploid egg cell. In the second instance, three conditions are expected in a 1:2:1 ratio; (1) two sex chromosomes and therefore male, (2) one sex chromosome and therefore female, and (3) no sex chromosomes.

No female parthenogenetic embryos have been observed, and it is difficult to assume that a condition corresponding to the normal female chromosome complement would be lethal. Reentrance of a polar body into the haploid egg can therefore probably be disregarded as an explanation of the findings reported in this paper.

Summary

The gonads of 67 parthenogenetic turkey embryos were studied microscopically after sectioning and staining with Heidenhain's Iron Hematoxylin and all were found to be male. The sex cords or seminiferous tubules and the "germinal epithelium" are present and typically male. Spermatogonia, Sertoli cells, and interstitial cells were observed in all gonads. All spermatogonia were found in the sex cords—a few observed in mitotic division. A mitochondrial crescent was observed in many spermatogonia from older gonads. Both right and left testes were present in all embryos. The intact gonads had the long cylindrical shape of normal embryonic testes. No female characteristics were observed.

The finding of males exclusively is discussed with respect to chromosome doubling, polar body reentry, and the sex chromosome relationship in the turkey.

Literature Cited

1. Stalker, H. D. Parthenogenesis in drosophila. *Genetics* 39:4-21. 1954.

2. Swift, C. H. Origin of the definitive sex-cells in the female chick and their relation to the primordial germ-cells. *Amer. Jour. Anat.* 18:441-470. 1915.

3. ————. Origin of the sex-cords and definitive spermatogonia in the male chick. *Amer. Jour. Anat.* 20:375-410. 1916.

4. Werner, O. S. The chromosomes of the domestic turkey. *Biol. Bull.* 61:157-164. 1931.

5. Willier, B. H. Specificity of sex, of organization, and of differentiation of embryonic chick gonad as shown by grafting experiments. *Jour. Exper. Zool.* 46:409-465. 1927.

6. Yao, T. S. and M. W. Olsen. Microscopic observations of parthenogenetic embryonic tissues from virgin turkey eggs. *Jour. Hered.* 46:133-134. 1955.

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CHROMOSOME BOTANY

IN 1945 Darlington and Janaki Ammal published their "Chromosome Atlas of Cultivated Plants." This work has recently appeared in a second edition issued in two volumes under different titles and with a change in authorship. The enlarged lists of chromosome numbers were compiled by Darlington and Wylie, embracing both cultivated and wild Gymnosperms and Angiosperms, and are treated under the more comprehensive title, "Chromosome Atlas of Flowering Plants." The theoretical material has been expanded and rewritten by Professor Darlington and now appears as a separate book.*

This theoretical discussion has as its purpose to "make it possible to bring our knowledge of wild and cultivated plants, our knowledge of systematics and ecology into one focus." Darlington emphasizes that the connection between chromosome studies and genetic principles is an intricate one and attempts to avoid this intricacy "by treating the matter to a large extent descriptively, and by pointing to the problems as much as by providing solutions."

The book is divided into seven chapters. Following them are two appendices. The first simply lists the earliest use in English of names for various cultivated plants. The second appendix, written by Professor E. B. Ford, points out the bearing of this book on the wide range of evolutionary problems in the Animal Kingdom.

There is a very brief description of cells and mitosis in which the nucleus is characterized as "the form in which the chromosomes

**Chromosome Botany*. C. D. Darlington. 186 + xii pp. Allen and Unwin, London (distributed by Macmillan, New York). 1956. $2.75.

exist between mitoses;" in other words, "the nucleus *is* the chromosomes." Chromosomes, sexual reproduction, meiosis, chiasma formation, inversions, interchanges and polyploidy are very sketchily outlined with some emphasis on misdivision and the origin of isochromosomes as a basis for a later consideration of intraspecific changes in chromosome number. The isochromosome of *Nicandra physaloides* and the difference in germination of 19- and 20-chromosome seeds are mentioned. Of considerable interest is the discussion of supernumerary chromosomes and the occurrence, origin and adaptive value of B chromosomes. The chromosome-counter is warned not to base counts of "species" on individual plants because of the variation in numbers of B chromosomes.

Darlington regards species as groups of plants which can be defined by their behavior; therefore, tests for their validity must depend upon experiment. He favors the Seventeenth Century definition of John Ray that " a species is a group of plants which breeds true from seed within its own limits." In emphasizing the dynamic aspects of systematics he criticizes taxonomists who are content to examine "dead plants, aided only by a dry lens" but he stresses insufficiently that most modern taxonomists are trained in the methods of the ecologist and cytogeneticist. Discussing the chromosomes, Darlington states that they reveal the patterns of variation in genera, the modes of origin of species and the kinds of reproduction of individuals. The evolutionary aspects of internal hybridity and reciprocal translocation, of auto-polyploidy, apomixis (said to be a widely occurring phenomenon), and the mutation selection of two genetic variants such as vegetative propagation and polyploidy are considered, and it is stressed that chromosome differences which distinguish related species are also found within species. Chromosome changes provide the materials out of which separate mating groups are built and establish genetic isolation.

Genes, chromosomes and environment are considered to be three independent and mutually selective variables. In some species genetic adaption to different ecological or geographical regions is the result of mutation or gene recombination, accompanied perhaps by very small chromosomal inversions or translocations, and all geographic differences must begin as genetic differences between plants growing in the same locality. Chromosome changes seem readily to facilitate the colonization of new territory. Polyploids appear to be especially well able to become adapted to new regions, and where there is aneuploidy, the basic chromosome number also seems to be increased as the plants migrate away from their earlier home. When reciprocal translocations are present larger rings appear at the periphery of the distribution of the taxon.

Evolution is described in terms of four levels of organization, the phenotype, the genotype, the arrangement of genes on the chromosomes, and the type of breeding system. Together they form a genetic system which is evolving because of changes in their relationships; and gene change is correlated with evolution at the three other levels. The importance of basic chromosome numbers, such as simple polyploidy and secondary or dibasic polyploidy, is discussed and the conclusion reached that "the polyploid species with the best expectation of life should be derived from crossing widely different diploid species." All cytogeneticists do not accept this conclusion, however. In a treatment of the origin of variations in the shapes and sizes of chromosomes and its significance in evolution it is pointed out that either the chromosomes of a given species are all roughly of about the same size and shape or show an even gradation of types or that the chromosome complement is bimodial consisting of both long and short chromosomes. Darlington states that in taxa in which the chromosomes are relatively stable there is evidence only of evolution during former geological epochs whereas in those with less stable chromosomes there is evidence of what is happening to races and species at the present time. He also states that chromosome numbers change much more in short-lived than in long-lived plants and that in woody plants basic chromosome numbers are usually fixed for many millions of years whereas in annuals this is true only for a few thousand generations; consequently, "the detailed systematics of many short-lived plants is completely fruitless unless combined with chromosome studies" whereas "intensive chromosome study of species and varieties of a woody genus is likely to be merely repetitive and equally unrewarding." It has been pointed out by others, however, that there is evidence of derived polyploidy in many woody forms.

The last three chapters are concerned with cultivated plants and their origin, and in them Darlington suggests that the sources of variation, the principles of selection and the conditions of geographical and genetic isolation operate equally in wild and in cultivated plants. Vavilov's theory that many staple crops show a series of centers of diversity is explained and his twelve centers of diversity of crops are listed along with the crops that probably arose in them. The recent ideas of Burkhill are also considered and it is mentioned that he "assumes that the areas in which crop plants arose were subject to the movements and aptitudes of men." Darlington states, "the reason for shift in the centre of diversity of crops is a shift in the centre of greatest cultivation." As factors in evolution by cultivation, unconscious selection in cultivation for tillage conditions, sowing conditions and harvesting conditions is considered. Maize is believed to have come from *Euchlena mexicana*, the annual teosinte, by selection, an origin which all botanists do not subscribe to.

Items to note are that when natural selection in a natural population is abandoned it must be replaced by permanent plant breeding using the whole apparatus of genetics and chromosome study, that cultivated plants "have given us a clearer view of the principles at work in the evolution of wild plants" and that the best understood plants are the ornamental plants as they have most recently been introduced into cultivation. The origin of eight cultivated plants is described in detail.

This book is well written and interesting. It is pregnant with ideas many of which, however, are stated too dogmatically and many of which will hardly receive universal acclamation. Too many statements are made without literature citation and one gets the idea that sometimes theories are bolstered by other theories rather than by facts. The frequent use of teleological expressions is quaint and the whole work is challenging and exciting whether one agrees or not with many of the statements.

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CORN AND ITS EARLY FATHERS

THE history of the improvement of any crop plant or domesticated animal is the story of a few perceptive men for whom the development constitutes both vocation and avocation. The recounting of the visions and activities of such men is always interesting. It becomes doubly so when the object of their attention is a plant as important to the economy as corn is to ours and for which many of us have inherited more than a trace of the mystical devotion shown by the American Indian. For him it was more than the staple of existence. Through centuries of dependence on the crop, the rituals connected with its cultivation became woven inextricably into the entire fabric of existence.

This short book, written in a most readable style and intended, at least partially, for popular consumption, has still much of interest for the reader whose acquaintance with the crop is more extensive than assumed by the authors in the introductory chapters. In fact, much of the discussion in the closing chapters seems aimed at the authors' colleagues in plant breeding work. From the account of the importance corn has among the Indians, the history moves smoothly and cohesively through the development of the remarkably efficient Corn Belt dent tynes by selection in inspired hands from crosses between the northern flint corns and their very dissimilar relatives, the soft southern dents. The men responsible were untrained in formal science, and some were relatively unlettered. As a basis for their efforts they had only a love for the crop and an intuitive perception of the path along which they must move. Yet the product of their combined efforts was so good that it took a major step in twentieth century science—the development of hybrid corn—to surpass it. For a general reading public, it might have been desirable to emphasize even more fully how dependent this process was on the intermingling of diverse types of corn even though they seemed unlikely parents. However, the inference is certainly present.

Interspersed with the later chapters depicting the development and spread of the superior midwestern dent varieties are those relating a quite different effort—the early (1877) attempts of Beal to increase vigor and yield by means of varietal crosses. In this he was quite obviously stimulated by Charles Darwin. This was the first attempt in maize to exploit the phenomenon of hybrid vigor. It was undoubtedly the intention of the authors to emphasize the intellectual debt owed to Darwin and to Beal by modern corn breeders. In view of the fact that the development of hybrid corn is compressed into one short and necessarily sketchy chapter, one is inclined to suspect that at least some of the non-scientific readers may be puzzled by the emphasis on the contributions of Darwin and Beal.

The closing chapters of the book strike most responsive chords in the reviewer. The thesis that the *sine qua non* of successful plant breeding is knowledge of one's material is hardly disputable. More controversial but equally valid is the view that the modes of statistical analysis now in vogue in applied genetics constitute sterile approaches to plant improvement. Finally the authors call attention to the fact that while a judicious mixing of diverse types of maize has given rise to a remarkable plant, a great deal of germ plasm remains to be sampled—germ plasm which contains our brightest hope for future improvement of this important crop.

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**Corn and Its Early Fathers.* Henry A. Wallace and William L. Brown. 134 pp. The Michigan State University Press, East Lansing, Michigan. 1956. $3.75.

THE CYTOLOGY OF TWO HYBRIDS OF GOSSYPIUM

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CYTOLOGICAL and genetical data have shown that the American cultivated cotton, *Gossypium hirsutum* L., is an amphidiploid. It has been demonstrated a number of times that one of the two genomes in amphidiploid cottons is similar to the genome (A) in the diploid Asiatic species, and the other is similar to the genome (D) in the diploid American wild species. The significance of this relationship has been pointed out by Brown and Menzel[5] who emphasized the importance of the American wild species for introducing new germ plasm into the highly selected varieties of American cultivated cottons.

All of the eight American wild species listed by Hutchinson *et al.*[7] have been crossed with *G. hirsutum*. However, only five of the hybrids have been examined cytologically. This report discusses the cytological relationships between *G. hirsutum* and two diploid American wild species, *G. aridum* (Rose and Standley) Skovsted† and *G. lobatum* Gentry‡.

Materials and Methods

Detailed taxonomic descriptions of *G. hirsutum* and *G. aridum* may be found in Hutchinson *et al.*[7]. More recently, Gentry[6] published a taxonomic treatment of *G. lobatum*. This is a new species, and is reported to be closely related to *G. aridum*. The type specimen of *G. lobatum* was collected in Michoacan, Mexico, which is within the region inhabited by *G. aridum*. *G. lobatum* was first found near the volcanic mountain Paricutin, hence it was earlier called *G. particutin*.

Following the terminology of Beasley[1] and Brown and Menzel[5], the symbols used to designate the genomes of the diploid American wild species and the amphidiploid in which their hybrids have been analyzed cytologically are as follows: *G. thurberi*, D_1; *G. armourianum*, D_{2-1}; *G. aridum*, D_4; *G. raimondii*, D_5; *G. gossypioides*, D_6; *G. hirsutum*, $(AD)_1$; and *G. barbadense*, $(AD)_2$. The subgenomes of *G. hirsutum* are designated as A_h and D_h.

The original crosses, using *G. hirsutum* as the female parent, were made in the tropical garden at Iguala, Mexico, and the hybrid seed were sent to this station by the Agricultural Research Service, U. S. Department of Agriculture. Material for cytological study was handled in the manner normally used in the iron-aceto-carmine smear technique.

Results and Discussion

Both hybrids are large shrubs under greenhouse conditions, and they bloom profusely most of the year. A comparison of some of the main morphological characters of the hybrids and the parents is given in Table I. Figure 6*A* and *B* are photos of a typical leaf, flower and bract of each hybrid.

G. hirsutum ($2n = 52$) × *G. aridum* ($2n = 26$)

Cytological analysis of chromosome pairing in hybrids between these two species was reported by Skovsted[11]. The pairing of the small D_h chromosomes of *G. aridum* with the small D_h chromosomes of *G. hirsutum* in Skovsted's and the present report (Figure 7*A* and *B*) shows that *G. aridum* belongs in the D genome group. In the present study, cytological data were collected from two hybrid plants and later combined, since there were no apparent differences in the two analyses. Chromosome association observed in the present hybrids was not markedly different

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†CB 1558

‡PEI 197676, CB 2811

***GOSSYPIUM HIRSUTUM* HYBRIDS**

Figure 6

A—Shows a typical leaf, flower and bracts of an F_1 hybrid of *G. hirsutum* × *G. aridum*. Note pronounced counterclockwise whorl of petals which is characteristic of the hybrid. *B*—Typical leaf, flower and bracts of an F_1 hybrid of *G. hirsutum* × *G. lobatum*.

from that observed by Skovsted (Table II); however, a higher frequency of trivalents was observed in the hybrids reported here. This may be due to the fact that four times as many pollen-mother-cells were analyzed in the present study than in the study by Skovsted. One cell had a maximum of five trivalents (Figure 7*A*). A higher frequency of trivalents would necessarily result in a lower frequency of bivalents and univalents in the present analysis than reported by Skovsted.

The trivalents were always composed of two D chromosomes and one A, (Figure 7*A* and *B*). No mention was made by Skovsted of this relationship, but from his figures it appeared that a similar association occurred in his material. Twenty-three (1.3 percent) of the 1908 bivalents consisted of AD chromosomes. Consequently, the frequency of AD pairing is slightly higher than indicated by the average of 1.16 trivalents per cell (Table II). A frequency of 1.16 trivalents per cell indicates a rather high homology between some of the A and D chromosomes. This homology could represent either residual homology, i.e., reflecting the homologous nature remaining between the two genomes, or it could represent interchange of segments between A_h and D_h chromosomes in *G. hirsutum*. It is not likely the latter since trivalents with this high frequency were not reported in several hybrids of *G. hirsutuum* with D species. Furthermore, the configurations of the five trivalents observed in a single cell would require that all five interchanges involve short segments, which seems improbable; also, there were no noticeable differences in arm length of the paired D chromosomes.

Brown[3] reported that at pachytene there is no noticeable difference in chromosome length of the paired A and D arms at this stage. However, the difference in chromosome length is apparent at metaphase I. In the trivalents of the present study the chiasma between the A and D chromosomes appeared always distal to the chiasma between the two D chromosomes when the latter chiasma was present. Brown (manuscript) has shown that there is apparently a difference in the rate of contraction of A and D chromosomes from pachytene to metaphase I. It is possible that the rate of contraction might affect the frequency and position of chiasma between A and D chromosomes, and that contraction might be least effective near the ends of the paired arms. If this is so, and the pairing of A and D chromosomes is the result of residual homology, which it seems to be, then a chiasma would most likely occur near the ends of the arms of A and D chromosomes. This is supported in the trivalents where the chiasma between A and D chromosomes appear in every case to be distal to the chiasma between the two D chromosomes.

Hexaploid and three-species tetraploid hybrids involving *G. hirsutum* and D species are usually fertile and pairing often occurs between the A and D chromosomes[4, 8]. Pairing and chiasma formation between two such chromosomes would result in an interchange in which the interchange would be near the ends of the arms. Such interchanges would

TABLE I. A comparison of main characters of *G. aridum*, *G. lobatum*, *G. hirsutum*, and hybrids

	$(AD)_1D_4$	D_4	$(AD)_1$	D_7	$(AD)_1D_7$
Leaf shape	1-5 lobed, broader than long, 8-11 cm wide, 9-12 cm long	entire, longer than broad, 4-8 cm wide, 5-11 cm long	3-5 lobed, broader than long, 11-19 cm wide, 5-10-15 cm long	3-5 lobed, broader than long, mostly 7-12 cm long, 8-14 cm broad	Mostly 3-5 lobed, broader than long mostly 11-16 cm long, 12-17 cm wide
Position of flower	sympodial	on peduncles 3-5 cm long	sympodial	Axillary in 2-3 distal nodes, 1-several a node	sympodial
Bracteoles	15-20 mm long, 5-6 teeth	2-3 mm long, entire	8-10 mm long, 11-13 teeth	4-5 mm long	30-40 mm long, 7-9 teeth
Calyx	9-10 mm long, tube 7 mm long, lobes 2-3mm long	?	9-10 mm long, tube 7 mm long, lobes 3 mm long	20-22 mm long, tube ca 12 mm, lobes 9-10 mm long	8-9 mm long, tube ca 6 mm, lobes 2-12 mm mostly 3 mm long
Corolla	6-6.5 cm long, pink with deep red spot	4-5 cm long, pink with deep red spot	5-6 cm long, pale cream, spotless	5-5.5 cm long, light pink with deep purple spot	6-6.5 cm long, light pink with deep red spot
Number of locules in ovary	3-4	3	4-5	3	3-4
Pollen	pale cream	pale cream	pale cream	pale cream	pale cream

be short and would likely pass unnoticed cytologically in later generations.

Beasley[1] and Brown and Menzel[4] observed a mean number of bivalents per cell in haploids of *G. hirsutum* of 1.7 and 1.5, respectively. The number of trivalents in the *hirsutum-aridum* hybrids is almost equal to the number of bivalents observed in the haploids. If the high frequency of trivalents in the hybrid reflects AD pairing normally observed in a haploid of *G. hirsutum*, then this might indicate a lesser degree of homology between the D_h and D_4 chromosomes. However, this is not the case since the two D chromosomes in a trivalent frequently had a chiasma in each arm in addition to the AD chiasma, and cells with 13 ring D bivalents were observed.

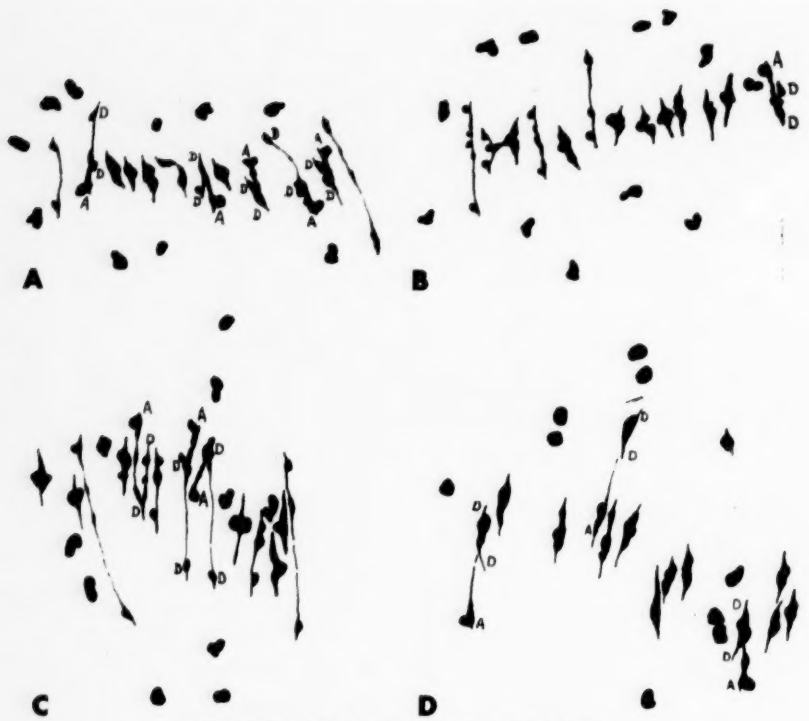
Eight quadrivalents were seen in the 156 pollen-mother-cells analyzed. Two quadrivalents were chains of A and D chromosomes, one of which had the arrangement of A-D-A-A and the other had the arrangement of A-D-D-A. The remaining six quadrivalents consisted only of D chromosomes. Five of the six had four arms joined by at least three chiasmata. It is possible that the association of four D chromosomes is due to interchange, most likely in *G. aridum*. The interchange probably is short since quadrivalents were infrequent.

Secondary association of A chromosomes was frequent. A one-chiasma bivalent involving two A chromosomes was observed.

In 29 cells having 13 small D bivalents and 13 large A_h univalents, the chiasma frequency per bivalent was found to be 1.75, which is the same as that observed by Skovsted[11]. An average of 9.80 bivalents per cell had at least one chiasma in each arm, and two cells out of the 29 showed that the 13 D bivalents had at least one chiasma in each arm. This is indicative that the D_h chromosomes and the *G. aridum* chromosomes have a great deal of homology. Furthermore, such close pairing shows that it may be relatively easy for blocks from each arm of the 13 chromosomes of *G. aridum* to be transferred by crossing-over into the D_h chromosomes of *G. hirsutum*. Such information on chromosome association in species hybrids of *Gossypium* is important to the breeder since it is indicative of the ease of transferring one or more characters from the wild species into cultivated cotton. If two or more of the wild species have similar characters of economic value, but one species shows a greater frequency of ring bivalents than others with *G. hirsutum*, the breeder then will know with which hybrid he is likely to have the most success in transferring the desired characters.

G. hirsutum × *G. lobatum* ($2n = 26$)

Since *G. lobatum* was found in Mexico and it is closely related to *G. aridum*, it is logical to assume that the species has chromosomes similar to the D genome group rather than to one of the other genome groups. Cytological analyses of three triploid hybrids



CHROMOSOME PAIRING IN F_1 HYBRIDS

Figure 7

Chromosome pairing at metaphase I in F_1 hybrids of *G. hirsutum* with *G. aridum* and *G. lobatum*. (A denotes chromosome of A genome, D denotes chromosome of D genome.) *A*—*G. hirsutum* × *G. aridum*, 7 bivalents + 5 trivalents + 10 univalents. *B*—*G. hirsutum* × *G. aridum*, 10 bivalents + 1 trivalent + 1 quadrivalent + 12 univalents. *C* and *D*—*G. hirsutum* × *G. lobatum*, 10 bivalents + 3 trivalents + 10 univalents. Note bivalents with one and two chiasmata, and trivalents as a chain or as a ring bivalent with an attached chromosome. ca. × 1,700.

shows that *G. lobatum* has 13 small chromosomes which pair with the 13 small D_k chromosomes of *G. hirsutum* (Figure 7*C* and *D*.) There were no apparent differences in the analyses of the three hybrid plants, so their data were combined in Table II. It can be seen that the average associations per cell is similiar to that observed in hybrids of the amphidiploids and other D species. The pairing behavior in the *hirsutum-lobatum* hybrids parallels to some extent the pairing in hybrids of *G. hirsutum* with *G. aridum* and *G. raimondii*. The similarity with the *hirsutum-aridum* hybrids might be expected since both D species are in the section Erioxyla.

The trivalents, as in *hirsutum-aridum* hybrids, were always composed of two D and one A chromosomes, but the frequency was much lower. Fifteen (0.80 percent) bivalents of the 1880 consisted of AD pairing. Consequently, the frequency of AD pairing is slightly higher than indicated by the 0.51 trivalents per cell. The discussion of trivalents under *G. hirsutum* × *G. aridum* would also apply here but perhaps not to the same extent since the frequency was lower. A maximum of four trivalents was observed in one cell. The types of trivalents observed in the *hirsutum-lobatum* hybrids appeared similar to those observed in the *hirsutum-aridum* hybrids.

Six quadrivalents were observed in the 143 pollen-mother-cells analyzed. Three of the six were chains involving A and D chromosomes as follows: A-D-D-A, A-D-A-D and the third in which an A chromosome was at-

tached to one arm of a D chromosome, and the other arm of the D was attached to another A and another D chromosome. The remaining three quadrivalents involved only D chromosomes, and consisted of two ring bivalents joined by a chiasma between an arm of each bivalent. This type of association of four D chromosomes was also most frequent in the *hirsutum-aridum* hybrids. If interchanges are responsible for quadrivalents in the *hirsutum-lobatum* hybrids, they must be short as in the *hirsutum-aridum* hybrids.

In 54 cells having only 13 small D bivalents and 13 large A_h univalents, the chiasma frequency per bivalent was calculated to be 1.76, which is the same as observed for the *hirsutum-aridum* hybrids in this study and the one by Skovsted[11]. The chiasma frequency is higher in the hybrids of *G. hirsutum* with *G. aridum* and *G. lobatum* than in other hybrids of *G. hirsutum* with D species (Table II). Whether this reflects a closer relationship of the chromosomes of *G. aridum* and *G. lobatum* with the D_h chromosomes of *G. hirsutum* is unknown. Segregation of marker genes in hexaploids of these hybrids might show if there is greater homology between the chromosomes of these two D species and the D_h chromosomes of *G. hirsutum* than between the chromosomes of other D species and the D_h chromosomes.

There was an average of 9.95 bivalents per cell with at least one chiasma per arm. This is similar to that observed in the *hirsutum-aridum* hybrids. A maximum of 12 ring D bivalents per cell was observed, but one cell, in which a trivalent was present, the 13 paired D chromosomes appeared to have at least one chiasma in each arm. Even though the frequency of ring bivalents per cell was nearly the same in both hybrids, it appears that the genome complement of *G. aridum* is more nearly like the D_h subgenome chromosomes of *G. hirsutum*. If there is a difference between the chromosomes of *G. aridum* and *G. lobatum*, when compared to the D_h chromosomes of *G. hirsutum*, the difference apparently resides in a few chromosomes, perhaps only one or two. Of the nine D species, *G. lobatum* seems to have morphological characters more in common with the amphidiploids.

It is obvious from the pairing of the 13 small chromosomes of *G. lobatum* with the 13 small D_h chromosomes of *G. hirsutum*, that *G. lobatum* belongs in the D genome group. The morphological characteristics of this species are sufficiently distinct from other D species, according to modern taxonomic procedures, to warrant specific rank. Therefore, it is proposed that *G. lobatum* be placed in a genome category of its own, D_7, even though hybrids with other D species have not been made and studied.

Summary

Cytological analyses are reported for F_1 hybrids of *G. hirsutum* ($2n = 52$) with *G. aridum* ($2n = 26$) and *G. lobatum* ($2n = 26$). *G. hirsutum* is an amphidiploid having the A and D subgenomes. *G. aridum* and *G. lobatum* are American wild diploid species having D genomes.

Comparisons are made of some morphological characters of the parents and hybrids. Chromosome conjugation at metaphase I of

TABLE II. Summary of average pairing in (AD)D hybrids

Genome Composition	II	III	IV	I	Xma freq. per bivalent	Authority
$(AD)_1D_1$	12.45	0.2		13.5	1.54	Skovsted[11]
$(AD)_1D_1$	12.05	0.5		13.4		Skovsted[11]
$(AD)_2D_1$	12.04	0.5		12.7		Skovsted[11]
$(AD)_1D_1$	12.70	0.18		13.06		Webber [12]
$(AD)_2D_1$	12.67	0.13		13.27		Ivengar[8]
$(AD)_1D_{2-1}$	12.15	0.4		13.5		Skovsted[11]
$(AD)_1D_{2-1}$	13.35	0.15		11.85		Skovsted[11]
$(AD)_1D_{2-1}$	12.7	0.05		13.45		Skovsted[11]
$(AD)_1D_{2-1}$	12.1	0.10		14.5	1.55	Skovsted[11]
$(AD)_1D_{2-1}$	12.0	0.30		14.1	1.49	Skovsted[11]
$(AD)_2D_{2-1}$	12.05	0.05		14.75		Skovsted[11]
$(AD)_2D_{2-1}$	12.0	0.6		13.2		Skovsted[11]
$(AD)_1D_4$	12.4	0.35		13.15		Skovsted[11]
$(AD)_1D_4$	12.0	0.6	0.05	13.0	1.64	Skovsted[11]
$(AD)_1D_4$	11.49	1.16	0.05	12.30	1.75	Author
$(AD)_2D_4$	12.28	0.15		13.99	1.73	Skovsted[11]
$(AD)_1D_5$	11.65	0.87	0.125	12.57		Boza Barducci & Madoo[2]
$(AD)_1D_6$	11.23	0.46		15.15		Brown & Menzel[5]
$(AD)_2D_6$	4.39	0.03		30.13*	1.11	Menzel & Brown[10]
$(AD)_1D_7$	12.15	0.51	0.04	13.00	1.76	Author

*Calculated by author.

the hybrids was similar to that observed in other hybrids of *G. hirsutum* with D species. In addition to bivalents and univalents, trivalents and quadrivalents were observed in the hybrids. Trivalents were much more frequent in the *hirsutum-aridum* hybrid than observed by other workers. Some implications of the trivalents and quadrivalents are discussed. It is concluded that AD pairing in the trivalents is due to residual homology, and that chiasma between the A and D chromosomes is probably in the terminal regions of the arms. The importance of determining the frequency of ring bivalents is discussed.

G. lobatum is a new species. Chromosome conjugation at metaphase I shows that it belongs to the D genome group. It is proposed that this species be placed in a genome category of its own, D_7.

Literature Cited

1. Beasley, J. O. Meiotic chromosome behavior in species, species hybrids, haploids, and induced polyploids of Gossypium. *Genetics* 27:25-54. 1942.

2. Boza Barducci, T. and R. M. Maldo. Investigaciones acerca del parentesco de la especie peruana de algodonero *Gossypium raimondii*, Ulbrich. *Estacion Experimental Agricola de la Molina, Lima, Peru. Boletin No. 22.* 1941.

3. Brown, Meta S. A comparison of pachytene and metaphase pairing in species hybrids of Gossypium. *Genetics 39* (Abs.):962-963. 1952.

4. ———— and Margaret Y. Menzel. Polygenomic hybrids in Gossypium. I. Cytology of hexaploids, pentaploids and hexaploid combinations. *Genetics* 37:242-263. 1952.

5. ———— and ————. The cytology and crossing behavior of Gossypium gossypioides. *Bull. Torrey Bot. Club* 79:110-125. 1952.

6. Gentry, H. C. A new Gossypium from Michoacan, Mexico. *Madrono* 13:261-265. 1956.

7. Hutchinson, J. B., R. A. Silow and S. G. Stephens. The Evolution of Gossypium. Oxford University Press. 1947.

8. Iyengar, N. K. Cytological investigations on hexaploid cottons. *Indian Jour. Agri. Sci.* 14:142-151. 1944.

9. Menzel, Margaret Y. and Meta S. Brown. The significance of multivalent formation in three-species Gossypium hybrids. *Genetics* 39:546-557. 1954.

10. ———— and ————. Isolating mechanisms in hybrids of Gossypium gossypioides. *Amer. Jour. Bot.* 42:49-57. 1955.

11. Skovsted, A. Cytological studies in cotton. IV. Chromosome conjugation in interspecific hybrids. *Jour. Genet.* 34:97-134. 1937.

12. Webber, J. M. Relationships in the genus Gossypium as indicated by cytological data. *Jour. Agri. Res.* 58:237-261. 1939.

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CORRIGENDUM

IN cooperation with two colleagues I recently published in this Journal an article entitled, Evidence of a New Lethal Gene In Cattle.* Several errors were inadvertently made in the final preparation of the pedigree network illustrating relations among the afflicted calves. This appeared as Figure 17, page 82, March-April, 1957. The errors and appropriate corrections are as follows:

The paternal Granddam of "A" is listed as 222,947 and should be 2,222,947. The Sire of "B" is 1,113,350 rather than 1,113,550; the Dam of this Bull 2,948,519 should be indicated in a circle rather than a square. Female 3,001,454 is sired by 930,694, also the Sire of "A", rather than by 930,696.

James F. Kidwell

University of Nevada
Reno

*Ternan, P. R., J. F. Kidwell and L. Walker. Evidence of a new lethal gene in cattle. *Jour. Hered.* 48:81-83. 1957.

THE MITOTIC CHROMOSOMES OF THE DOMESTIC FOWL

EARL H. NEWCOMER*

THE fowl has been the subject of an enormous amount of embryological, physiological and genetic investigation from which much data have accumulated but its chromosomes are still widely considered to be the least known among the vertebrates[23]. Genetic evidence has demonstrated five autosomal and one sex linkage groups but the chromosome number is still in doubt, as may also be the identity of the sex chromosome. Reasons for this seem to have been the technical difficulties in securing adequate cytological preparations, the limitations of study to largely metaphase plates and, not least, the interpretation of such data within the confines of presumed cytogenetic dicta. Thus actual counts reported in the literature[3] vary from a low of 12 to upwards of 80 with a mode around 40. Most investigators have only attempted to estimate the number but Yamashina[45] reported the precise figures of 77 in the female and 78 in the male and insisted that the chromosomes were constant in number and morphology in all of the 17 breeds investigated by him.

As to the identity of the sex chromosome, three of the larger chromosomes have been variously associated with sex determination, but following the work of Suzuki[35] and Unger[38], the metacentric, fifth largest chromosome is generally accepted because it is reported single in the female and paired in the male. Genetic evidence has long demonstrated the heterozygosity of the female for sex and the digamety is thus assumed to be the XO-XX type.

In addition to the identity of the sex chromosome, there is one other area of general agreement concerning the chromosomes of the fowl. It is that the chromosomes consist of two categories, the six pairs of large chromosomes called the macro-chromosomes and the smaller micro-chromosomes whose numbers are reported as either variable or uncountable by all but Yamashina.

In a recent study of the spermatogenesis of the fowl[27] with a new killing fluid[28] and the squash technique we concluded there are six pairs of euchromosomes corresponding to the macro-chromosomes plus a variable number of heterochromatic elements which we loosely designated as accessory chromosomes and which correspond to the category of the micro-chromosomes of previous investigators. The difference, though drawn from technical grounds, implied a qualitative genetic distinction between the two categories.

The present study is an extension of our previous work to the mitotic chromosomes with the hope of clarifying to some extent the enigma of fowl chromosome cytology.

Methods and Materials

The Columbian breed was chosen for use on the basis of availability but only after a comparative study with the Rhode Island Red breed used in our previous study showed no discernible differences in their somatic chromosomes.

After examining tissue cultures—whole 40-hour embryos, the lungs of sexed eight-day embryos and the gonads of 10-13 day old embryos—we chose the last for study on the basis of being easily sexed, prolific of mitoses and being possibly freer from the criticism of somatic chromosome irregularities reported in other animals[2, 37, 40].

The technique of preparation was similar to that used in our previous study save that the common difficulty in get-

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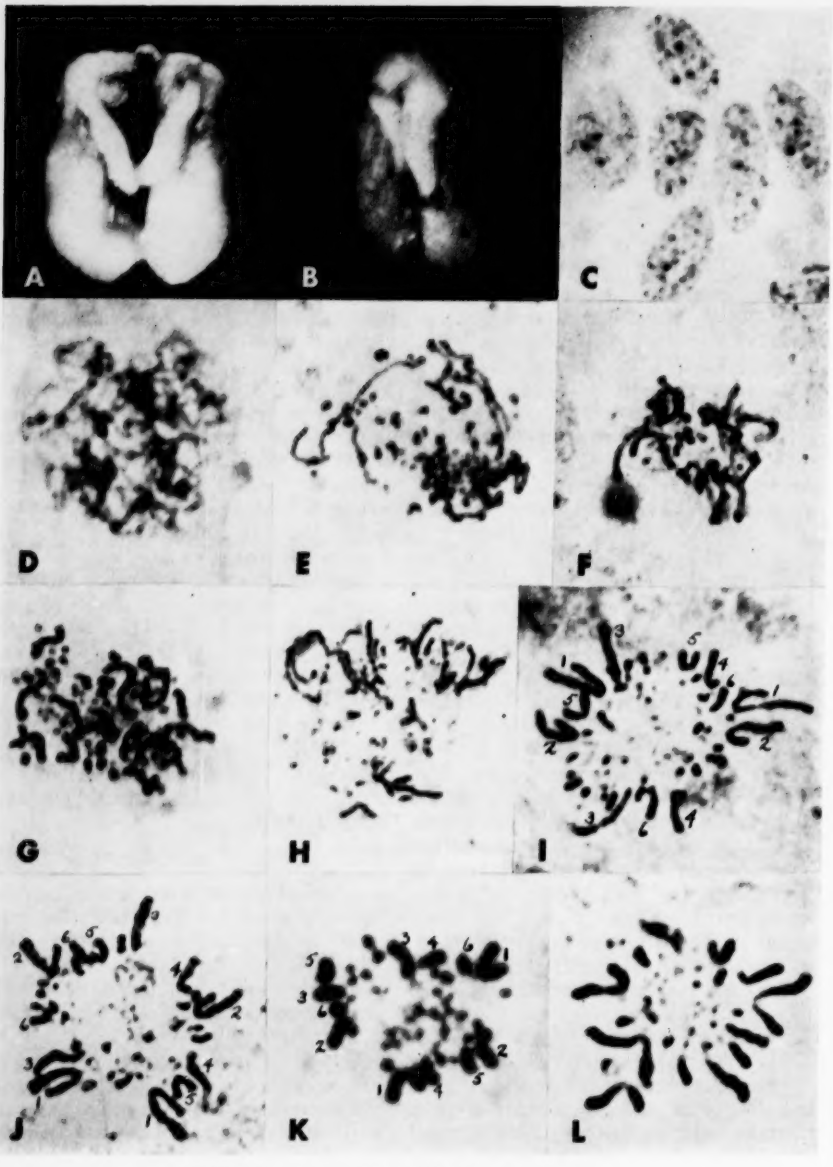
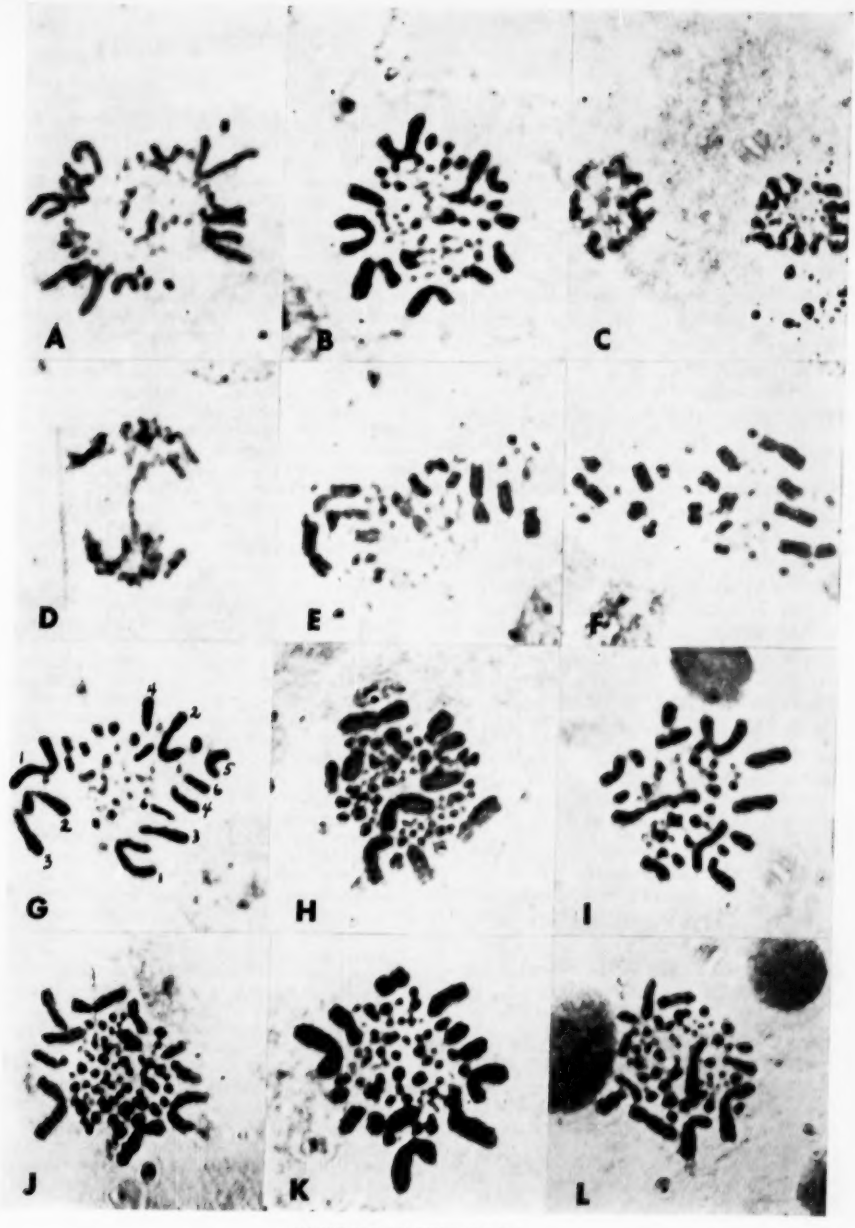


MITOTIC CHROMOSOMES IN THE FOWL

Figure 8

Figures 8 and 9 are photomicrographs of smears from gonads of 11 to 13 day-old embryos unless otherwise stated. *A* and *B*—Gonads of 8½ day-old embryos on mesonephros of male and female respectively. *C*—Interphase nuclei. *D*—Early prophase showing appearance and chromosome association of chromocentric heterochromatin. *E*—Later prophase indicating apparent origin of chromatoid bodies from chromocenters and their partial incorporation within the chromosomes. *F*—Prophase with nucleolus and associated chromosome. *G*—Late prophase with chromosomes and variably heterochromatic chromatoid bodies. *H*—Late prophase with two nuclelar associated chromosomes at upper left. Some chromosomes with heterochromatic banding. *I*—Labelled metaphase from a 40-hour male embryo. Note morphological difference in first pair. *J*—Labelled chromosomes of male with heterochromatic regions in 1, 2, 3, 4. *K*—Contracted metaphase chromosomes with chromatoid bodies showing fusion and fragmentation. *L*—Metaphase of 40-hour embryo showing heterochromatic regions in chromosomes and associated chromatoid bodies.



MITOSIS IN THE FOWL

Figure 9

A—Metaphase from 40-hour male embryo showing morphological variations between homologs. *B*—Metaphase after pre-treatment with paradichlorobenzene. *C*—Telophase daughter nuclei. Nucleus at right shows typical allocycly of chromosomes and chromatoid bodies. *D*—Late anaphase showing quantitative separation of fused heterochromatic chromatoid bodies. *E* and *F*—Metaphase after pre-treatment with colchicine for two hours. Note typical C-mitotic appearance and kinetochores of chromosomes and diminution in volume and number of chromatoid bodies. *G*—Metaphase after water pre-treatment. *H*—Metaphase after pre-treatment in versene. Compare number and size of chromatoid bodies in *G* with *H*. *I*—Metaphase of male after pre-treatment in versene and distilled water for 10 minutes. *J-L*—Metaphases after water pre-treatment. In *L*, note nuclei freed from cytoplasm.

ting well separated chromosome configurations made necessary various pre-treatments of the tissue prior to fixation. Following the suggestions of Hughes[16], Hsu and Pomerat[15] for spreading chromosomes, we pre-treated tissues in a series of dilute Gey's nutrient solutions from half strength down to and including distilled water. It was subsequently discovered that Makino and Nishimura[21] had previously used a pre-treatment in distilled water for 10 minutes at room temperature and this was used successfully. The great advantage of the extreme hypotonicity is that it causes an osmotic burst of the cytoplasm, liberating clean, free nuclei and division figures accompanied by a separation of the chromosomes which can be sharply stained and observed (Figure 9*J-L*).

In addition to pre-treatment in distilled water, gonads were sliced and incubated at 39° C. for periods of 15 minutes to three hours in half strength Gey's nutrient solution to which either versene, paradichlorobenzene[25], coumarin[5] or colchicine were added. Concentrations of 0.1 percent versene, 0.02-0.5 percent colchicine, paradichlorobenzene and coumarin to saturation were found effective and incubation periods of two to three hours seemed optimum.

After fixation in Newcomer's isopropyl mixture[28], tissues were smeared and stained in either propionic carmine or Feulgen in which the staining seemed to be identical.

For comparison, similar tissues with and without pre-treatments were fixed in Hermann's, Flemming's and Bouin's fluids, processed by the paraffin technique and stained with either Haidenhain's haematoxylin, crystal violet, Feulgen or with two modifications[19,36] of Unna's methyl-green, pyronin technique.

Observations

Technique

White[43] recently observed, "Thus the student of avian speciation must, for the present, resign himself to the fact that he cannot use the method of comparative cytology, which has proved so valuable in other groups, simply because the technical obstacles do not permit us to arrive at an absolutely accurate morphological description of the chromosome set of any species of bird. . . ." With few exceptions, all of the observations on fowl chromosomes have been obtained from sectioned tissues fixed with salts of heavy metals and most recorded observations were confined to metaphase plates. The technical difficulties referred to above by White are caused by the large number of elements and their compact association at metaphase which prevents adequate simultaneous examination of all members. This has been augmented by the apparent addiction to slowly penetrating fixing fluids such as Hermann's, Flemming's and Bouin's, which, though excellent for most cytological work, only enhance the difficulties here by clumping the chromosomes. The mordanting effect of these heavy metal solutions and the use of mordanted haematoxylin as a stain has also obscured their heterochromatic nature. Furthermore, the exclusive attention of most investigators to metaphase plates prevented an adequate examination and interpretation of the so-called micro-chromosomes. It is interesting and perhaps significant that most of the early workers who essayed a smear technique or worked with tissue cultures[13,29] reported variations in number of microchromosomes due to their fusion or fragmentation and sometimes questioned the status of these elements. Having also used the sectioning techniques of previous investigators in the present study, this writer can emphatically concur with White as to their inadequacy for avian chromosomes.

The micro-chromosomes

In our study of spermatogenesis[27], the basic chromosome number of the fowl was reported to be six and the micro-chromosomes were relegated to the status of accessory or supernumerary chromosomes on the basis of their variability in structure, number and behavior. Melander[24] defined accessory chromosomes "to denote such extra chromosomes as are not homologous with those of the ordinary complement and whose number is in equilibrium in a population." White[43,44] objected to the term accessory chromosome as having been loosely used by some authors in reference to the sex chromosome or polysomy and suggested the term supernumerary for the same definition, which does seem preferable. The present study of the mitotic chromosomes, which are always more favorable for morphological study, has widened the genetic and cytological rift between the micro- or supernumerary and macro-chromosomes by removing the former from the category of chromosomes. These elements, by their apparent origin, structure, behavior and function do not fit any accepted definition of any kind of chromosome. To describe them, this writer has proposed the term chromosomoid.

Apparent origin, structure, behavior and function of the chromosomoids

At the onset of prophase, conspicuous but

scattered masses of chromocentric heterochromatin appear in the nucleus (Figure 8*D*). As prophase proceeds, part of the heterochromatin may become as discrete as small chromosomes while some assume a sticky, depolymerized appearance and are associated with the developing matrix of the prophase chromosomes (Figure 8*E-G*). The prophase chromosomes themselves are variably heterochromatic at this stage and their association with the chromocentric heterochromatin is such as to suggest physical incorporation of the latter by the former (Figure 8*G* and *H*). In this connection, Hughes[17] has observed: "It is known that large heterochromatic aggregates are liable to be broken down into smaller units, each of which appears to form a part of an individual chromosome." As prophase culminates, there is a redefinition of the chromosomoids into more discrete forms (Figure 8*G*) and here their number has been observed to vary from 25 to 56 units of varying sizes and staining intensities. It is often difficult or impossible to separate chromosomoids from chromosomes either here or at some metaphases due to fusion forms. The usual distinction of the chromosomes occupying the periphery of the polar metaphase plate and the chromosomoids filling the central area does not always hold (Figures 8*I* and *J*) and since kinetochores cannot be discerned on any of the subterminal chromosomes without pre-treatment, the larger fusion forms of chromosomoids have been counted as chromosomes to the confusion of all. These larger fusion forms may also carry negatively heteropycnotic areas resembling kinetochores (Figure 8*H*) and the smaller allocyclic dividing chromosomoids can appear as pairs of chromatids (Figure 8*G* and *K*). It is also not unusual to observe many of the chromosomoids at metaphase to be connected in an anastomosing series which in turn are often associated with some of the chromosomes (Figure 8*K* and *L*). This might appear to be a fixation artifact but all fixatives used produce it and it has been reported as well by previous investigators[29].

Judging from observed frequencies, the anaphase stage of mitosis must be extremely rapid. The chromosomoids may achieve anaphase separation and migration either precociously or belatedly (Figure 9*D*). In the latter they produce fused, heterochromatic bridges and in either event it seems clear that their division is not precise as befitting chromosomes, but fortuitous.

In the general depolymerization of chromatin at telophase, the chromosomoids disappear first, often leaving the 11 or 12 chromosomes peripherally located in the nucleus in a discrete and deeply stained condition (Figure 9*C*).

Perhaps the most convincing evidence for the de-novo origin, evanescent status and non-chromosomal nature of the chromosomoids is obtained from their reaction to pre-treatment prior to fixation, especially with the drug colchicine. As is well known, colchicine inhibits spindle formation and prevents the anaphase migration of the chromosomes but does not interfere with the chromosome cycle of nucleic acid synthesis and division which results in ploidy. The drug also produces the so-called C-mitotic effect, i.e., the chromosomes appear shorter with parallel chromatids and the kinetochore is rendered conspicuous.

Pre-treatment of chick tissues for several hours in colchicine serves thus to distinguish the chromosomes from the chromosomoids and also suggests a function for the latter. Figure 9*E* and *F* represent typical C-mitotic metaphase plates after colchicine in which the 11 chromosomes of a female are shown with a markedly reduced number of chromosomoids which suggests their being utilized as reserves for chromatid replication. The fluctuation in numbers and size of chromosomoids throughout mitosis in different tissues may thus be explicable in terms of the mitotic index and degree of their utilization for chromosome duplication. This suggested function is also supported by their behavior during early prophase and their disappearance at the end of meiosis prior to spermatid formation[27].

The macro-chromosomes

Since the so-called micro-chromosomes of the fowl have been relegated to a status of chromosomoids, the prefix macro for the six pairs of chromosomes of the fowl is redundant and will be omitted.

The most striking and puzzling observation of the mitotic chromosomes is their failure to observe the so-called mitotic constants at metaphase. It is often impossible to establish the identity of the homologous pairs on the basis of measurement (Figures 8*I*, *K*, *L*, and 9*A*, *I*). The classical reglarity of these chromosomes depicted in the drawings of previous investigators has rarely been observed in this study and this allometry was common with all techniques. Crew[6] and Hance[13] observed a similar pleomorphism but it is not limited to an extreme capacity for contraction and elongation (Figure 8*I* and*K*) nor is it due to pre-treatment. This confusion has been reflected in the incorrect use of the terms V shaped, J shaped and metacentric with reference to the first, second, fourth and fifth largest chromosomes, all but the fourth of which has been identified as the sex chromosome because presumably it is unpaired. Thus Guyer[9], Crew,[6] Hance[12], Akkeringa[1], White[42] and Popoff[32] all assumed the largest chromosome to be unpaired in the female and hence identified with sex determination.

The variations in relative sizes of homologous chromosomes and relative lengths of their respective arms seems to be associated with the variable amounts of intercalry heterochromatin and also possibly to differences in degree of internal and relational coiling of the chromatids (Figure 8*H-L*). It appears as though the normally high rate of nucleic acid synthesis during prophase is being supple-

mented by the imbibition of partially or entirely depolymerized nucleic acids from the chromosomoids (Figure 8*E*, *G* and *H*). The pleomorphism of the chromosomes and the fluctuations in number and volume of the chromosomoids also suggest some such relationship, varying with the mitotic index.

In addition to the individual variations in morphology of the chromosomes, there often appears to be a variation in number. It is frequently possible to count more than 11 or 12 elements of chromosomal size at metaphase (Figures 8*I*, *L* and 9*G*) but pre-treatment suggests that this is due to the fusion of the chromosomoids (Figure 9*E*, *F*, *H* and *K*) into elements of chromosomal size. Versene, colchicine, paradichlorobenzene, coumarin and distilled water all tend to stabilize the morphology of the chromosomes and, by revealing their kinetochores, aid in distinguishing chromosomes from chromosomoids.

The sex chromosome and the nucleolus

Although the general polymorphism of the untreated somatic chromosomes may beguile one into believing almost any of the six pairs of chromosomes are unpaired at times, the fifth largest metacentric chromosome appears most consistently in an apparently unpaired condition in the female at metaphase (Figures 8*I*, 9*A* and *G*). There is substantial unanimity among the investigators now concerning this point but, as will be discussed later, there is some genetic evidence which suggests a larger chromosome and also the possible presence of a Y chromosome in the female. The chromosome cytology of the fowl is not yet sufficiently definitive to eliminate these possibilities.

Gates[6] has presented evidence from insects and mosses for the relationship between the sex determining chromosome and the nucleolus and thinks the phenomena may be of general occurrence. Unfortunately, the nucleolus in the mitotic nuclei of the fowl seldom persists beyond early prophase and, though chromosome associated (Figure 8*F* and *H*), it was impossible to identify the chromosome. There also appears to be no satellite involved in the association of nucleolus and chromosome and no satellited chromosomes can be identified in the mitotic complement. The chromosome most often appearing to be satellited is the long, sub-terminal third chromosome (Figure 8*I* and *L*), but the ubiquity of the chromosomoids and their frequent terminal juxtaposition with the chromosomes often produce the appearance of satellites. Whichever chromosome is associated with nucleolar formation, the region of association is characterized by the localized positive pycnosis of the chromosome (Figure 8*F* and *H*).

Discussion

The evolutionary origin and significance of chromosomoids can only be a matter for speculation at the present time. Melander[24] has suggested that "the so-called micro-chromosomes of certain animals may also be the origin of accessory chromosomes." Although possessing some of the attributes of chromosomes, they lack the definitive structure and precision of chromosome behavior throughout the nuclear cycle and, as such, would seem to constitute an extension to the spectrum of known chromatic entities in the nucleus. Being heterochromatic, they presumably share the properties and functions of heterochromatin and as such, are probably not without genetic significance. Since the size of the chromocentral mass of a pre-prophase nucleus is a measure of the heterochromatin of a species, the chromosomoids apparently constitute the bulk of heterochromatin. Mather[22], Goldschmidt[8], Pontecorvo[31], Hannah[14], Vanderlyn[39] and others have reviewed the evidence for the function of heterochromatin and there is general agreement that heterochromatin is not genetically inert as once supposed. There is substantial support from genetic evidence that it carries what Mather[22] has called polygenes: i.e., genes without major effect but whose effects are minute but cumulative and thus modifying in action. This interpretation would seem to find support from genetic data from the fowl. From Hutt's book[18], one learns that body size, egg production, disease resistance and most other physiological characters of economic importance are not Mendelian in inheritance but are due to an accumulation of factors and are pleiotropic in expression. There is also an impressive prominence of such terms in the genetic literature of the fowl as epistasis, hypostasis, penetrance, multiple alleles, incomplete dominance, complimentary genes, pleiotropy, etc., which suggests the presence of some rather general modifying mechanism in the germ plasm of the fowl. The mechanism could well be the functioning of the chromosomoids. This postulate at least provides an experimental basis for further, and perhaps definitive, work in the direction of understanding some of the enigmatic genetics of the fowl.

One other aspect of the possible modifying action of the chromosomoids upon the genetics of fowl may be considered. If, as seems cytologically demonstrated, the polymorphism of the mitotic and presumably also the meiotic chromosomes is caused largely or in part by the intercalary insertion of variable amounts of heterochromatin, some of the disparate linkage data might be explicable from this cause. The physical effects of increasing or changing gene distances on the chromosome could produce changes in the recombination index and/or position effects, thus further modifying inheritance. Whether the functions of the heterochromatic chromosomoids could be expanded to include Lerner's concept of homeostasis[20] is perhaps a matter for speculation. But polygenic balance, multifactorial inheritance and continuous variation are all nuances of the possible expressions of hetero-

chromatin and by experimental alteration of the latter with new techniques, further information may be obtained.

The confirmation of our previous results[27] in establishing the chromosome number of the fowl to be $2n = 12$, gives it the lowest chromosome number of all the vertebrates, save the marsupials. Since the number of linkage groups is equal to the haploid chromosome number, it would seem that there are six linkage groups in the fowl. This agrees with present genetic data. The fact that there are known genes in the fowl which segregate independently of any of the known linkage groups does not necessarily argue for more linkage groups as has been recently assumed[4]. This can be as plausibly explained by the limitation of our knowledge of linkage data or the disparate results of some of these studies.

Van Brink and Ubbels[4], on the basis of a limited study of somatic metaphase plates, have not confirmed our designation of a non-chromosomal status for the so-called micro-chromosomes of the fowl and rather startlingly concluded that the determination of the chromosome number of the fowl is beyond the possibilities of cytology. A study of their plates and drawings, however, would seem to confirm the non-chromosomal status of their micro-chromosomes. They appear allocyclic, acentric, variably heterochromatic, variable in number and to range in size from chromosomal to the limits of microscopic resolution. Sokolow and Trofimow[34] made similar observations long ago and ascribed it to polysomy and fragmentation. No known definition of chromosome will fit these elements and it seems clear that they belong to a different category or order of nuclear constituents. While many of their properties are shared with various types of accessory chromosomes, they show no evidence of possessing either diffuse, polycentric, or localized kinetochores and their addiction to fusion and fragmentation further diminishes their chromosomal status although they share the general property of ectopic pairing in meiosis with certain heterochromatic chromosomes[33]. We therefore suggest the term chromosomoid, which means chromosome like, to describe them until further experimental studies may either confirm or change their status.

In our previous study of spermatogenesis, it was observed that in view of Warren's[41] linkage studies in which reported sex-linked characters showing independent assortment and which he ascribed to multiple cross-overs, we questioned on cytological grounds the suitability of the fifth largest, metacentric chromosome for being associated with sex determination. In view of its short arms and low chiasma frequency, it seemed the least likely of the complement to satisfy the requirements. Pontecorvo[30] has also reported a case of a partially sex linked dominant in the Jungle Fowl female with a supposed recombination value of about 20 percent. This behavior presumes the presence of a Y chromosome in the female. Despite the many reports of an unpaired fifth chromosome in the female fowl, the cytology of this point is not yet clear. One does not always find the conspicuously single fifth chromosome in mitotic metaphase plates and further studies should be made on this point. One may ascribe the failure to observe the unpaired metacentric in most metaphases to either technique or the general polymorphism of the mitotic chromosomes, but this explanation may not be completely satisfactory. Since cytologic and genetic data are at odds concerning this, it may be premature to conclude the digamety of the fowl to be of the XX, XO type. There are other possible modes of sex determination and it seems presumptuous to insist upon one which is at variance with genetic data, especially in view of the history and present status of fowl cytology. The possibility of having overlooked a small homologue of the metacentric fifth chromosome in the welter of small elements in the nucleus or of autosomal participation in sex determination should still be considered. A thorough study of oogenesis or of a sex reversed female by a smear technique should help to establish this point. Miller's early study[26] is not convincing and should be repeated with modern techniques.

Summary and Conclusion

The mitotic chromosome complement of the domestic fowl apparently consists of 12 chromosomes in the male and 11 in the female. Genetic data suggests the re-examination of the mode of sex inheritance and technical cytological limitations may not warrant the conclusion of the present X-O type of digamety assumed for the female. The somatic chromosomes exhibit an unusual degree of polymorphism at metaphase, apparently caused by the interstitial incorporation of variable amounts of heterochromatin.

By means of tissue pre-treatment prior to fixation with colchicine, versene, coumarin, paradichlorobenzene and water, it is shown that the so-called microchromosomes are not chromosomes but structures described as and named chromosomoids. The chromosomoids are heterochromatic and may constitute reserve nucleic acid for chromosome replication. Their heterochromatic properties may also constitute a quantitatively modifying mechanism in the germ plasm of the fowl.

Literature Cited

1. Akkeringa, L. J. Die chromosomen bei einigen Hühnerrassen. *Zeit. f. mikr.—anat. Forsch.* 8: 325-342. 1926.

2. Beatty, R. A. How many chromosomes in mammalian somatic cells? *Int. Rev. Cytology* III: 177-197. Academic Press. N. Y. 1954.

3. Brant, J. W. A. A review of literature on chromosome studies of the fowl. *Poul. Sci.* 31: 409-417. 1952.

4. Van Brink, J. M. and G. A. Ubbels. La question des heterochromosomes chez les Sauropsidés. Oiseaux. *Experientia.* 12: 162-164. 1956.

5. Cornman, I. The responses of onion and lily mitosis to coumarin and parasorbic acid. *Jour. Exp. Biol.* 23: 292-297. 1947.

6. Crew, F. A. E. A case of non-disjunction in the fowl. *Proc. Roy. Soc. Edinburgh* 53: 89-100. 1933.

7. Gates, R. R. Nucleoli, satellites and sex chromosomes. *Nature* 144: 794-795. 1939.

8. Goldschmidt, R. B. Heterochromatic heredity. *Proc. 8th Int. Cong. Gen.* 244-255. 1948.

9. Guyer, M. J. Studies on the chromosomes of the common fowl as seen in the testes and in embryos. *Biol. Bull.* 31: 221-268. 1916.

10. Hance, R. T. The somatic chromosomes of the chick and their possible sex relations. *Science* 59: 424-425. 1924.

11. ————. The chromosomes of the chick soma. *Biol. Bull.* 51: 443-447. 1926.

12. ————. A comparison of mitosis in chick tissue cultures and in sectioned embryos. *Biol. Bull.* 50: 155-159. 1926.

13. ————. Sex and the chromosomes in the domestic fowl. *Jour. Morph. and Physiol.* 43: 119-145. 1926.

14. Hannah, A. Localization and function of heterochromatin in *Drosophila melanogaster. Advances in Genetics* 4: 87-125. 1951.

15. Hsu, T. C. and C. M. Pomerat. Mammalian chromosomes in vitro. II. A method for spreading the chromosomes of cells in tissue culture. *Jour. Hered.* 44: 23-29. 1953.

16. Hughes, A. Some effects of abnormal tonicity on dividing cells in chick tissue culture. *Quar. Jour. Micro. Sci.* 93: 207-219. 1952.

17. ————. The Mitotic Cycle. Academic Press. N. Y. 1952.

18. Hutt, F. B. Genetics of the Fowl. McGraw-Hill Book Co. New York. 1949.

19. Kay, E. R. M. A Mixture of methyl green and pyronin for cell fraction studies. *Stain Tech.* 28: 41-44. 1953.

20. Lerner, I. M. Genetic Homeostasis. John Wiley & Sons. New York. 1954.

21. Makino, J. and I. Nishimura. Water pre-treatment squash technic. *Stain Tech.* 27: 1-7. 1952.

22. Mather, K. The genetical activity of heterochromatin. *Proc. Roy Soc. London. B* 132: 308-332. 1944.

23. Matthey, R. Les Chromosomes des Vertebres. Librairie de l'university, F. Rouge. Lausanne. 1949.

24. Melander, Y. Accessory chromosomes in animals, especially in *Polycelis tenuis. Hereditas* 36: 19-38. 1950.

25. Meyer, J. R. Prefixing with paradichlorobenzene to facilitate chromosome study. *Stain Tech.* 20: 121-125. 1945.

26. Miller, R. A. Spermatogenesis in a sex-reversed female and in normal males of the domestic fowl, *Gallus domesticus. Anat. Rec.* 70: 155-183. 1938.

27. Newcomer, E. H. and J. W. A. Brant. Spermatogenesis in the domestic fowl. *Jour. Hered.* 45: 79-87. 1954.

28. Newcomer, E. H. A new cytological and histological fixing fluid. *Science* 118: 161. 1953.

29. Painter, T. S. and L. J. Cole. The genetic sex of pigeon-ring dove hybrids as determined by their sex chromosomes. *Jour. Morph.* 72: 411-439. 1943.

30. Pontecorvo, G. Meiosis in the striped hamster and the problem of heterochromatin in mammalian sex chromosomes. *Proc. Edinburgh Roy. Soc. B.* 62: 32-42. 1943.

31. ————. Structure of heterochromatin. *Nature* 153: 365-367. 1944.

32. Popoff, W. W. Uber die zahl der chromosomen und uber die heterochromosomen des haushühnes. *Zeit Zellf. u. mikr. Anat.* 17: 341-346. 1933.

33. Slizynski, B. M. Ectopic pairing and the distribution of heterochromatin in the X-chromosome of salivary gland nuclei of *Drosophila melanogaster. Proc. Edinburgh Roy. Soc.* 62: 114-119. 1943-47.

34. Sokolow, N. H. and J. E. Trofimow. Individualität der chromosomen und geschlechtsbestimmung beim haushühn. *Zeitschr. ind. Abstamm. u. Vererb.* 65: 327-352. 1933.

35. Suzuki, I. On the chromosomes of the domestic fowl (in Japanese). *Zool. Mag.* 42: 358. 1930.

36. Taft, E. B. The problem of a standardized technic for the methyl-green-pyronin stain. *Stain Tech.* 26: 205-212. 1951.

37. Timonen, J. and E. Therman. Variation of the somatic chromosome number in man. *Nature* 166: 995-996. 1950.

38. Unger, H. Beitrag zur chromosomenforschung der Vogel. *Zeits. f. Zellforch. u. mikr. Anat.* 25: 476-500. 1936.

39. Vanderlyn, L. The heterochromatin problem in cytogenetics as related to other branches of investigation. *Bot. Rev.* 15: 507-582. 1949.

40. Walker, B. E. and E. R. Boothroyd. Chromosome numbers in somatic tissues of mouse and man. *Genetics* 39: 210-219. 1954.

41. Warren, D. C. Linkage relations of autosomal factors in the fowl. *Genetics* 34: 333-350. 1949.

42. White, M. J. D. The chromosomes of the domestic chicken. *Jour. Genet.* 26: 345-350. 1932.

43. ————. Chromosomes of the vertebrates. A review. *Evolution* 3: 379-381. 1949.

44. ————. Animal Cytology and Evolution. Cambridge University Press. New York. 2nd Ed. 1954.

45. Yamashina, M. Y. Karyotype studies in birds. I. Comparative morphology of chromosomes in seventeen races of domestic fowl. *Cytologia* 13: 270-296. 1944.

GENETIC RELATIONSHIP AND REACTIONS TO SKIN GRAFTS

JAMES V. CRAIG AND L. J. HIRSCH*

IT has been demonstrated by Loeb and Wright[4], Loeb, King and Blumenthal[3], Kozelka[2] and Blumenthal[1] among others that the severity of the immune reaction resulting from tissue transplantation is associated with the genetic relationship of donor and host. Most of the investigators used subjective scores based on external graft appearance, length of graft survival or histological examinations. Blumenthal[1], however, used the increase of lymphocytes in the circulating blood following tissue transplantation as an indicator of the severity of the reaction.

The experiments reported here were conducted to determine the extent to which a technique modified from that of Blumenthal[1] and involving skin grafts with chickens would supply quantitative data indicating the association of relationship coefficients and severity of reaction. Limited evidence was gathered to indicate whether grafts between individuals of different breeds resulted in greater reactions than those between unrelated individuals of the same breed.

Material and Methods

Three trials were carried out at the Illinois Station in 1954-55. Breeds, number of chicks, age at grafting and the intervals between grafting and post-operative differential white cell counts are indicated in Table I. Relative increments of lymphocytes calculated as the ratio of the percentage of lymphocytes present post-operatively to the percentage present pre-operatively in the circulating blood were used as an indication of the severity of the immune reaction.

Chicks were anaesthetized with Nembutal injected either intraperitoneally or intravenously, and fitted grafts of approximately 2 cm. diameter were exchanged between individuals of known relationship. A Barred Columbian (C) strain developed from an initial breed cross and mated *inter se* for several generations prior to this experiment was

TABLE I. Breeds, numbers of chicks, age at grafting and intervals between pre- and post-operative differential white cell counts

Trial No.	Breeds* of Donor	Breeds* of Host	No. of Chicks	Age of Chicks	Interval between Pre- and Post-operative Differential Counts
1a	C	C	16	3 wks.	3 days
1b	C	C	14†	3 wks.	5 days
2	C	C	16	5 wks.	5 days
3a	C	C	16	5 wks.	5 days
3b	RIR × C	C	8	5 wks.	5 days
3c	RIR	C	8	5 wks.	5 days

*Breeds coded as follows: C = Barred Columbian; RIR × C = Rhode Island Red × Barred Columbian; and RIR = Rhode Island Red.

†Two chicks died between the three and five day post-operative counts.

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used to study the skin-grafting reaction between individuals of known relationship coefficients. This strain was also used to supply host birds receiving skin grafts from Rhode Island Red by Barred Columbian (RIR × C) crossbred and Rhode Island Red (RIR) donors. All grafts were sutured in place on the outer median thigh and covered with a film of collodion.

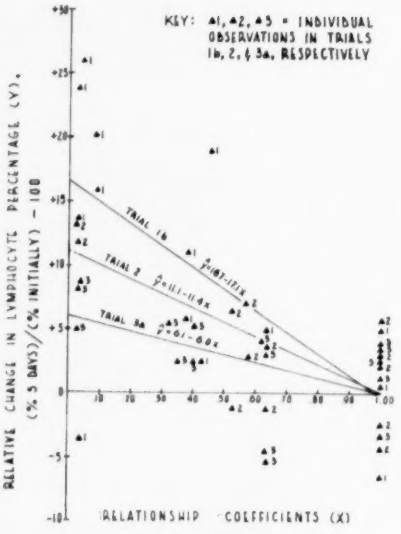
Homografts involved chicks ranging in relationship coefficients from 0.02 to 0.63. Autotransplants represented the 1.00 degree of relationship.

Differential white cell counts were made from blood smears taken immediately before the operation and at three and/or five days post-operatively. In each instance two smears were made per bird and these were stained with Giemsa's stain. All differential counts were made by the same person (L. J. Hirsch).

Results

Relationship of donor and host was found to be negatively associated with the severity of the immune reaction to transplanted skin, Table II and Figure 10. In each of the three trials significant associations were found. On the basis of limited data from the first trial it appeared that lymphocyte increases on the fifth day after grafting were more closely correlated with relationship coefficients than were those from the third day. Subsequent post-operative readings were therefore made on the fifth day only.

In the third trial, C chicks served as recipients to grafts from C, RIR × C and RIR chicks, with results as shown


KEY: ▲1, ▲2, ▲3 = INDIVIDUAL OBSERVATIONS IN TRIALS 1b, 2, & 3a, RESPECTIVELY
RELATIVE CHANGE IN LYMPHOCYTE PERCENTAGE (Y), (% 5 DAYS)/(% INITIALLY) − 100
TRIAL 1b
TRIAL 2
TRIAL 3a
RELATIONSHIP COEFFICIENTS (X)

CHANGES IN LYMPHOCYTE PERCENTAGES

Figure 10

Scatter diagram, showing relative changes in lymphocyte percentages of hosts following skin grafting from donors with varying degrees of genetic relationship.

in Table III. All C donor to C host reactions were adjusted by means of regression to a basis of zero relationship so that comparisons would be for the equivalent of immune reactions between unrelated individuals within the C strain vs. reactions involving individuals of other breeds as donors. Since the RIR × C chicks had one C parent it was expected that transplants from them would cause a lower response than those from

TABLE II. Associations of genetic relationship of donor and host and severity of the immune reaction as indicated by lymphocyte increases

Trial No.	Breeds of: Donor	Host	Regression of Lymphocyte Increase on Relationship	Correlation of Lymphocyte Increase and Relationship
1a	C	C	−0.13	−0.39
1b	C	C	−0.17*	−0.64*
2	C	C	−0.11**	−0.73**
3a	C	C	−0.06*	−0.55*

*Significant at the 5 percent level.
**Significant at the 1 percent level.

the RIR when placed on C hosts. Although the RIR × C grafts caused a slightly larger response than C to C grafts the difference was not significant. RIR grafts on C hosts resulted in significantly greater responses than C to C grafts.

Discussion

The significant association found between relative lymphocyte increases in the circulating blood of chicks receiving skin transplants from donors of known relationship indicates the suitability of the technique used for obtaining biological estimates of the genetic similarity of individuals within experimental populations. The minor surgery involved with skin grafting and the quantitative nature of the measurements are desirable features of the technique.

The increased response obtained when skin grafts were received from donors of a different breed as compared to grafts from donors of the same breed adjusted to zero relationship equivalent indicates that no plateauing of effect was obtained at this level of genetic diversity.

Using the technique as described here it should be possible to check calculated relationship coefficients in experimental populations in which inbreeding has been practiced. A control population maintained without inbreeding from the same foundation stock used in the production of the inbreds could well serve as a basis for such comparisons.

Another possible use of the technique would be to test the hypothesis that heterotic effects, measured as deviations from the mid-parent means, may be predicted on the basis of the intensity of homograft reactions. The genetic diversity of the stocks to be crossed could be estimated by the severity of the reaction.

A word of caution in the application of this technique appears justified. The responses obtained between reciprocal donors and hosts of the same breed in this study were symmetrical. However, Loeb and Wright[4] have shown that asymmetrical results between reciprocal donors and hosts may occur. They found that the F_1 between two inbred lines accepted grafts almost as well as autografts whereas the parent inbred lines showed large reactions to grafts received from the F_1. Thus the foreign genes of the donor's graft not possessed by the host appeared to be responsible for the reaction. This asymmetry of response would not be expected except in such special cases in which reciprocal donors would not possess approximately equal proportions of genes foreign to the recipients.

Summary

Significant associations between relative increases of lymphocytes in the circulating blood following skin transplantation and relationship coefficients were found in three trials. Greater responses were obtained when grafts were made between breeds of chickens than for grafts within a breed.

Literature Cited

1. Blumenthal, H. T. Effects of organismal differentials on the distribution of leukocytes in the circulating blood. *Arch. Path.* 27: 510-545. 1939.

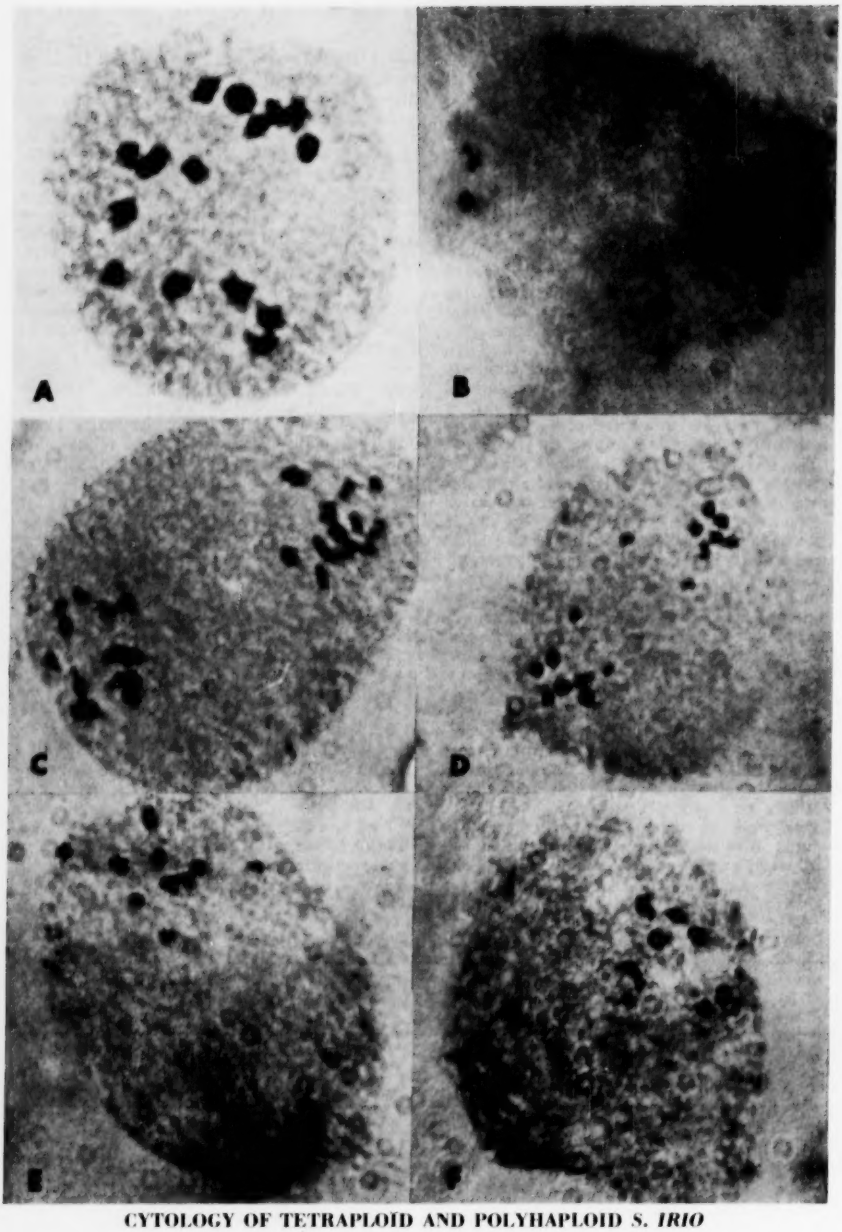
2. Kozelka, A. W. Integumental grafting as a means of analyzing the factors determining the secondary sexual characters of the Leghorn fowl. *Jour. Exp. Zool.* 61:431-484. 1932.

3. Loeb, L., H. D. King and H. T. Blumenthal. Transplantation and individuality differentials in inbred strains of rats. *Biol. Bul.* 84:1-12. 1943.

4. Loeb, L. and S. Wright. Transplantation of tissues in hybrids of inbred families of guinea pigs and the individuality differentials. *Amer. Jour. Path.* 3:251-283. 1927.

TABLE III. Relative lymphocyte increases resulting from skin grafts within and between breeds

Trial No.	Breeds of: Donor	Host	No. of Hosts	Relative Increase (%) of Lymphocytes in the Circulating Blood
3a	C	C	16	6.1 ± 0.86
3b	RIR × C	C	8	6.8 ± 2.51
3c	RIR	C	8	10.4 ± 1.76



CYTOLOGY OF TETRAPLOID AND POLYHAPLOID *S. IRIO*

Figure 11

A and *C*—Tetraploid. *B*, *F-H*—Polyhaploid. *A*—Fourteen bivalents and one nucleolus can be seen at diakinesis. *B*—Metaphase I with 14 univalents. There was no significant primary or secondary pairing at this stage. *C*—Anaphase I, with 14-14 distribution. *D-F*—Anaphase I; *D*—6+2/2; *E*—6+2/2, 6+4/2; *F*—4+4/2.

A POLYHAPLOID PLANT OF THE TETRAPLOID RACE OF SISYMBRIUM IRIO

T. N. Khoshoo*

SISYMBRIUM IRIO L. is a very common winter annual of the Northwestern Indian subcontinent. Previously, Khoshoo[5] reported that the species is polytypic and contains four cytological races (*2x*, *4x*, *6x* and *8x*) and one sterile triploid hybrid which arises spontaneously every year. So far only the diploid ($2n = 2x = 14$) has been reported from Europe (Manton[13], Darlington and Wylie[2]).

In April 1953 the writer noted a plant which was morphologically very much like the subcaulis type (Khoshoo[6,7]) of the tetraploid race but this plant was totally sterile. It grew in a somewhat dry spot among a small population of subcaulis plants. The dry season had advanced and the plant had practically stopped all active growth. However, the writer managed to get an idea about its somatic chromosome number from the developing sepals. The number was found to be in the neighborhood of 14 but not more than that. Therefore, the possibility of its being a spontaneous triploid hybrid ($2n = 3x = 21$) was ruled out both on morphological and on cytological grounds. On the other hand both these criteria were strongly suggestive of its being a haploid of the tetraploid race. To be exact this plant is referred to as a polyhaploid using the terminology of Katayama[4]. It is hardly necessary to indicate the importance of this plant in the interpretation of the genome relations and the phylogeny of the tetraploid race of *S. irio*.

Methods

Only one fixation of the flower buds was possible after which the plant was transferred to the garden. Soon the aerial portion dried and was cut off in order to save the two small buds which had appeared near the ground level. Due to the advancing dry weather even these died and all attempts to keep the root alive failed.

The flower buds were fixed in acetic-alcohol (1:3) in which the acetic acid component was saturated with iron acetate (Marks[14]). Pollen grains were examined in aceto-carmine—glycerine mixture.

Observations

Morphological

A detailed morphological comparison of the subcaulis type (Figure 12*A*) with a polyhaploid (Figure 12*B*) was not possible because the latter was discovered rather late in the season and by the time its cytological nature was determined, it had practically dried.

In general it may be said that the polyhaploid was diminutive (Figure 12*B*) being hardly 21 cms. tall and the surrounding subcaulis plants (Figure 12*A*) were about 35-50 cms. in height. A comparison of the two illustrates the similarity of their branching pattern. In the polyhaploid all floral parts were much smaller than the normal plants. The siliques were very small (0.5-1 cm.), deformed and totally empty. In strong contrast to this the siliques of the tetraploid were about 3-3.4 cms. long and full of seeds.

The polyhaploid continued flowering for a longer time than the surrounding subcaulis plants. This was apparent from the fact that practically all the subcaulis plants had stopped flowering much earlier and were drying and shedding seeds when the polyhaploid was still flowering. The total sterility perhaps acted as the stimulus for the continued flowering.

Cytological

Tetraploid

At diakinesis 14 bivalents were always noted accompanied by 1-2 nucleoli (Figure 11*A*). So far over 2,000 pollen-mother-cells from about 200 plants have been examined and not a single multivalent association has been discovered. At anaphase I the bivalents disjoin neatly (Figure 11*C*) which in turn gives rise

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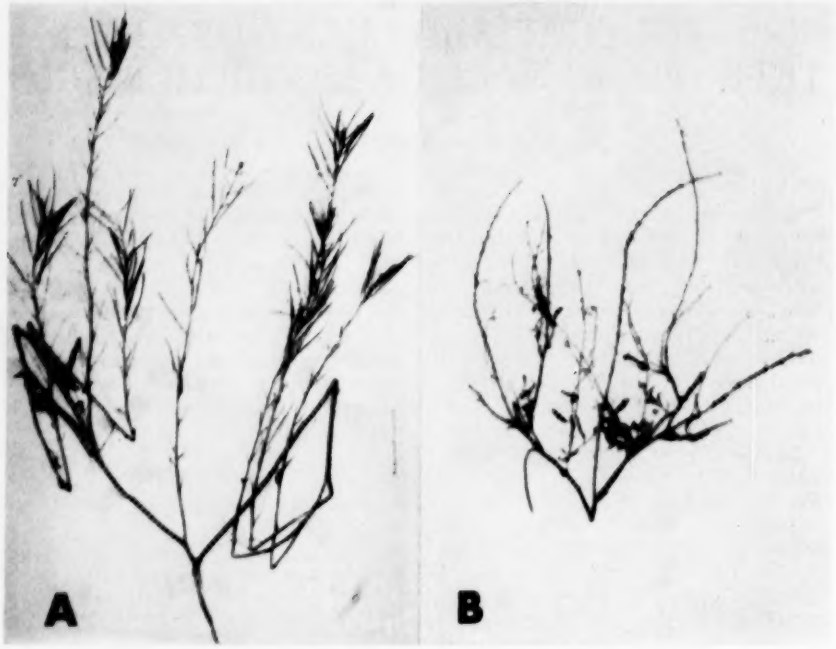

TETRAPLOID AND POLYHAPLOID *S. IRIO*

Figure 12

A and *B* show the growth habits of the tetraploid and polyhaploid races of *S. irio* respectively. The similarity of their branching habits can be noted. All floral parts were much smaller in the polyhaploid and the siliques were very small, deformed and totally empty.

to a normal telophase II. Tetrads are normal and the percentage of good pollen ranges from 94-100.

Polyhaploid

No cells at the pachytene stage were available. The earliest stage observed was what appeared to be metaphase I since there was no nucleolus in the pollen-mother-cells. Ten such cells were seen. In eight of these there were 14 univalents (Figure 11*B*) showing no significant primary or secondary pairing whatsoever. Of the remaining two cells, one had one bivalent + 12 univalents and the other had three bivalents + eight univalents. The bivalents were a little longer than those in the tetraploid. It was not possible to determine whether the bivalents of the polyhaploid involved chiasma formation. The univalents were about half the size of the bivalents of the tetraploid (compare Figure 11*B* with 11*A*).

If the pairing in the 10 cells examined represents the prevailing situation in the polyhaploid then 80 percent of the cells contained 14 univalents and naturally exhibited no pairing whatsoever. On the average each cell contained 0.4 bivalents and 13.2 univalents.

The univalents were scattered in the cell. It was therefore difficult to distinguish between metaphase I and anaphase I particularly when there were no bivalents so that a metaphase plate was not organized. However, a stage at which the univalents occupied two poles and some were in a process of division was interpreted as anaphase I. Forty cells were seen at such a stage. The univalents were in one, two or three groups.

The most interesting arrangements were those in which the sum total of the chromosomal bodies exceeded the somatic number of 14. A closer examination of such cells revealed that varying numbers of univalents had divided. The number of divided univalents range from one to six (Figure 11*D-E*). The univalents divide in only about 27.5 percent of the cells at anaphase I.

One cell (Figure 11*F*) showed a peculiar arrangement. It contained six univalents on

either pole and in the middle there was a bivalent. Evidently in this case the univalents had segregated and the bivalent was still undivided. Similar arrangements have been observed by Kostoff[9]. Ordinarily it is the bivalents that disjuct first and are followed by univalents[3].

In 85 percent of the cells two interphase nuclei were organized at telophase I. These were either equal or unequal in size. In 13 percent of the cells there were three interphase nuclei and in the remaining 2 percent there were two interphase nuclei accompanied by one to three abnormally stretched thin univalent bridges extending from one pole to the other. According to Oestergren[16] such thin chromatin threads are actually a part of the kinetochore 'subjected to deformation'. Such bridges have been observed in the haploid of rape[17], rye[10] and sugar beet[11].

Only five cells could be analyzed during metaphase II. In three cells there were seven chromosomes in each plate, in another cell there were three metaphase figures with 6, 4 and 4 chromosomes and in the fifth cell there were only two metaphase figures with 7+½ and 6 chromosomes. The remaining one chromatid was in the general cytoplasm.

At telophase II there were four equal or unequal nuclei in 80 percent of the pollen-mother-cells. In five percent of the cells there were five nuclei and in the remaining 15 percent there were six nuclei.

The pollen grains are very small, empty and completely sterile. The size of these grains is smaller (4.4-13.2 μ) than even the sterile pollen of the triploid (13.2-17.6 μ). The total pollen sterility of the polyhaploid can be explained not only by the inequality in number of chromosomes in the various telophase II nuclei but in particular by the random segregation of chromosomes. The latter results in unharmonious combinations even though a nucleus may contain the monoploid number of seven chromosomes. Ordinarily total pollen sterility is expected to exist in true haploids and polyhaploids of the genomic alloploids. However, the haploid of *Mathiola* (Lesley and Frost[12]) is an exception. In this case dyads resulted in 70 percent of the cells due to the regular division of univalents at anaphase I.

The polyhaploid did not yield any seed.

Conclusions

Nothing definite can be said about the origin of the polyhaploid. It may have arisen through haploid parthenogenesis due to either temperature shock or twin-seedlings and does not appear to be the result of any hybridity. Perhaps the cause may be high temperature and it is pertinent to mention that Müntzing was able to secure a haploid of rye by heat treatment (cf. Nordenskiold[15]).

The meiosis of the polyhaploid clearly shows that there is virtually no pairing. The mean number of bivalents per cell was 0.4. If this bivalent frequency was due to any extensive homogenetic association, then there should have been a corresponding quadrivalent frequency in the tetraploid. As mentioned previously over 2,000 pollen-mother-cells of the tetraploid race did not reveal any multivalents. Instead it is reasonable to assume that this pairing was perhaps due to the fusion of heterochromatic ends as has been shown to be the case in haploids of rye and sugar beet by Levan[10, 11]. Kostoff[8] also suggested that the pairing in haploid einkorn wheat is due to heterochromatin and does not indicate any homology, though Smith[8] does not agree with him. It is equally possible that the pairing in the polyhaploid indicates homology of very minute segments. Such homology may be strong enough to manifest itself especially when there is a general lack of competition for pairing among chromosomes. Furthermore, in the tetraploid, because of the strong preferential pairing, this homology of the minute segments is never expressed in the form of quadrivalents.

The polyhaploid is also to be viewed as the F_1 sterile hybrid that gave rise to the tetraploid race by doubling. Therefore it could be concluded that the two genomes of this diploid hybrid have virtually no homology. Furthermore, the hybrid has in all probability only chromosomal sterility since the tetraploid race is fully fertile. The biosystematic relationship of the parents of this F_1 hybrid is therefore cenospecific. It is now a well known fact that successful genomic alloploids arise after such intercenospecific crossing[1, 19, 20]. The question of the exact parentage and the biosystematic status of the tetraploid race will be discussed in detail in a subsequent paper.

Summary

A polyhaploid ($2n = 14$) of the tetraploid race ($2n = 28$) of *Sisymbrium irio* Linn. was discovered in April 1953. A study of over 2,000 pollen-mother-cells of the tetraploid persistently revealed 14 bivalents. On the other hand 80 percent of the cells of the polyhaploid showed 14 univalents. On the average there were 0.4 bivalents and 13.2 univalents per cell. Total pollen and seed sterility resulted, due to irregular meiosis. It has been concluded that the small amount of pairing in the polyhaploid is due either to the fusion of heterochromatin or to the homology of very minute segments. The latter is perhaps strong enough to manifest itself in a general lack of competition for pairing. In the tetraploid this homology is never expressed due to the strong preferential pairing.

The polyhaploid is also the F_1 sterile hybrid which gave rise to the tetraploid race by doubling. From its meiosis it is apparent that the biosystematic relationship of the parents of the tetraploid is at a cenospecific level. Furthermore, the sterility in this F_1 hybrid is only chromosomal.

Literature Cited

1. Clausen, J., D. D. Keck and W. M. Hiesey. Experimental studies on the nature of species. II. Plant evolution through amphiploidy and autoploidy with examples from Madiinae. *Car. Inst. Wash. Publ.* 564. 1945.
2. Darlington, C. D. and A. P. Wylie. Chromosome Atlas of Flowering Plants. G. Allen and Unwin Ltd. London. 1955.
3. Gajewski, W. On the behaviour of univalents at meiosis in some *Geum* hybrids. *Hereditas* 35:221-241. 1949.
4. Katayama, Y. Karyological comparisons of the haploid plants from octoploid *Aegilotriticum* and diploid wheat. *Jap. Jour. Bot.* 7:349-380. 1935.
5. Khoshoo, T. N. Biosystematics of the *Sisymbrium irio* Complex. *Nature.* 176:608. 1955.
6. ————. Biosystematics of *Sisymbrium irio* complex. I. Modifications in phenotype. In press.
7. ————. Biosystematics of *Sisymbrium irio* complex. II. Morphology of the races. In press.
8. Kostoff, D. Heterochromatin at the distal ends of the chromosomes in *Triticum monococcum*. *Nature* 141:690-691. 1938.
9. ————. The problem of haploidy. *Bibl. Genet.* 13:1-144. 1942.
10. Levan, A. Studies on meiotic mechanism of haploid rye. *Hereditas* 28:177-211. 1942.
11. ————. A haploid sugarbeet after colchicine treatment. *Hereditas* 31:399-410. 1945.
12. Lesley, M. M. and A. B. Frost. Two extreme "small" *Mathiola* plants: A haploid with one and a diploid with two additional chromosome fragments. *Amer. Nat.* 58:22-33. 1928.
13. Manton, I. Introduction to the general cytology of the Cruciferae. *Ann. Bot.* 45:509-566. 1932.
14. Marks, G. E. A controllable carmine technic for plants with small chromosomes. *Stain Tech.* 27:333-336. 1952.
15. Nordenskiold, H. Studies on haploid rye plant. *Hereditas* 25:201-210. 1939.
16. Oestergren, G. The mechanism of the co-orientation in bivalents and multivalents. *Hereditas* 37:85-156. 1951.
17. Olsson, G. and A. Hagberg. Investigations on haploid rape. *Hereditas* 41:227-237. 1955.
18. Smith, L. Haploidy in Einkorn. *Jour. Agric. Res.* (Wash.). 73:291-301. 1946.
19. Stebbins, G. L., Jr. Types of polyploids: Their classification and significance. *Advances in Genetics* 1:403-429. 1947.
20. ————. Variation and Evolution in Plants. Columbia Univ. Press. New York. 1950.

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COMPLEMENTARY GENES FOR BLOOM IN *RICINUS COMMUNIS* L.

Leroy H. Zimmerman*

BLOOM in the castorbean plant *Ricinus communis* L. consists of a white waxy substance on both the vegetative and reproductive parts of the plant. The presence or absence of bloom was reported by White[6], Harland[1], Peat[5], and Patwardhan[4] to be due to a single gene with bloom dominant or partially dominant over no-bloom. The symbols *B* and *b* were proposed by Harland[1] to denote the genes responsible for the presence and absence of bloom respectively. Different types of bloom were reported by the latter three authors and were described in terms of the amount of bloom on various parts of the plant. In every case it was shown that this variability was due to modifier genes, since their expression was dependent on the presence of the *B* gene. Narain[3] has recently reported additional modifications of the bloom character, with their genetic explanation to be given later.

In 1952, the F_1 of the cross U.S. 70 × U. S. 121-6 was observed to have bloom. This was unusual since both parents had none. The F_2 had 78 plants with bloom and 55 with no-bloom, a good fit of a 9:7 ratio. This ratio would obviously suggest that dominant com-

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plementary genes were involved. To test this assumption further the cross was repeated. The results of this and other crosses made primarily for other purposes are given in Table I. It will be noted that all of the no-bloom varieties whose F_1's showed the bloom character gave segregations in the F_2 which fit a 9:7 ratio. It will also be noted that crosses between certain no-bloom varieties, Nebraska 145-4 × U.S. 121-6 and U.S. 74 × U.S. 70, did not have bloom in the F_1. As might be expected, the varieties of each cross which had no-bloom in the F_1 were of similar origin and those which had bloom were of different origin. Crosses of no-bloom varieties with Cimarron and 3/441-9, which breed true for the bloom character, gave the expected bloom in F_1 and a 3:1 segregation for bloom and no-bloom in the F_2 (See Table I).

In the F_2 of Nebraska 145-4 (no-bloom) × 3/441-9 (bloom), bloom was found to be linked with the *M* gene for reddish stem color. The following dihybrid segregation was obtained:

B M	*Bm*	*bM*	*bm*
255	10	15	84

This ratio is clearly a deviation from the 9:3:3:1 expected for this cross. The calculated recombination value using the product method[2] is 7 ± 1.4 percent which is close to the 8.3 percent value reported by Harland[1] for the same characters. Thus the gene for no-bloom carried by Nebraska 145-4 and the *b* gene reported by Harland[1] are probably the same. The data then suggest the following genotypes for the varieties:

	Genotype	*Phenotype*
U.S. 121-6	bbB_tB_t	No-bloom
Nebraska 145-4	bbB_tB_t	No-bloom
U.S. 74	BBb_tb_t	No-bloom
U.S. 70	BBb_tb_t	No-bloom
Cimarron	BBB_tB_t	Bloom
3/441-9	BBB_tB_t	Bloom

The suggested symbols for the newly discovered gene pair is B_tb_t.

Literature Cited

1. Harland, S. C. Inheritance in *Ricinus communis* L. Part I. *Jour. Genet.* 10:207-218. 1920.

2. Immer, F. R. and M. T. Henderson. Linkage studies in barley. *Genetics* 28:419-440. 1943.

3. Narain, A. Bloom character in castor oil plant (*Ricinus communis* L.) *Current Science* 21:166-167. 1952.

4. Patwardhan, C. B. A preliminary note on inheritance in castors. *Jour. Indian Bot. Soc.* 10:100-109. 1931.

5. Peat, J. E. Genetic Studies in *Ricinus communis* L. *Jour. Genet.* 19:373-389.

6. White, O. E. Inheritance studies on castor beans. *Brooklyn Botanic Garden Memoirs* 1:513-520. 1918.

TABLE I. Data on the presence or absence of bloom and its inheritance in F_1 and F_2 of several castorbean varieties and their crosses

Variety or Cross	Generation	Number in Classes			Chi-square		P
		Bloom	No-bloom	Total	3:1	9:7	
U. S. 121-6			Breeds true				
U. S. 70			Breeds true				
N-145-4			Breeds true				
U. S. 74			Breeds true				
Cimarron		Breeds true					
3/441-9		Breeds true					
U. S. 121-6 × U. S. 70	F_1	All					
U. S. 70 × U. S. 121-6	F_1	All					
U. S. 70 × U. S. 121-6	F_2	78	55	133		0.310	>0.50
U. S. 70 × U. S. 121-6	F_2	193	159	352		0.158	>0.50
U. S. 74 × U. S. 121-6	F_1	All					
U. S. 121-6 × U. S. 74	F_1	All					
U. S. 121-6 × U. S. 74	F_2	115	103	218		1.084	>0.20
N-145 × U. S. 121-6	F_1		All				
U. S. 74 × U. S. 70	F_1		All				
N-145-4 × U. S. 74	F_1	All					
Cimarron × U. S. 121-6	F_1	All					
Cimarron × U. S. 121-6	F_2	166	50	216	0.395		>0.50
U. S. 70 × Cimarron	F_1	All					
U. S. 70 × Cimarron	F_2	149	59	208	1.256		>0.20
N-145-4 × Cimarron	F_1	All					
N-145-4 × 3/441-9	F_1	All					
N-145-4 × 3/441-9	F_2	265	99	364	0.938		>0.30

NOTCHED EARS IN NEW ZEALAND DAIRY CATTLE

M. A. MacDonald*

AN inquiry from the farming public regarding the possibility of an inherited traumatical aural abnormality drew attention to the prevalence of naturally occurring "nicked" or "notched"† ears in some lines of New Zealand dairy cattle.

Natural notched-ear conditions have been reported previously in three widely separated geographical areas. Yamane[5] reported it in Ayrshire cattle which were descendants of an animal exported to Japan from California. This condition could be traced in turn to an Ayrshire bull, Eglington 21, which was described on importation to the United States from Scotland in 1859 as "dark brown and white with nicked ears." Yamane's search of early U. S. Ayrshire records indicated that with one exception all listed notched-ear cattle (five bulls and eight cows) were descendants of this bull. The origin of this peripheral aural abnormality is obscured by the fact that herd book records do not go back as far as the date of importation. The notch varied markedly in size and distinct types were categorized. In some animals (Figure 13) the ears were slightly notched; in others notches reduced the ears to approximately one-half normal size. In the latter case the ridges of the inner surface of the lobe were developed excessively. The degree of indentation remained proportionally constant throughout life. The character described by Yamane[5] was shown to be homozygous dominant (DD) in deeply notched animals and heterozygous dominant (DR) in slightly notched animals; being transmitted as an incomplete dominant in full accordance with Mendelian principles. Snyder[3] reported what appears to be a similar form of notched-ear in Ohio Ayrshires indicating that the character had no effect on the animals' production of meat or milk but disqualified it for show purposes. No mention of the possibility of mutual ancestry with the animals described by Yamane was made by Snyder.

NOTCHED EAR TYPES
Figure 13

Sketches of a hereditary notch in ears of Ayrshire cattle. *A* Shows a normal ear; *B* a small notch and *C* a large notch. Typical notches observed in New Zealand appear in broken outline. Those reported by Yamane[5] are superimposed and appear in solid outline.

A distinctly different notched-ear condition was reported in Texas by Lush[1]. It was found among the descendants of two registered Jersey bulls. The condition was not breed specific. It was manifest as a missing portion of the lower edge of the ears. Both ears were not always notched to the same extent in an animal and there was variation between animals in degree of notching. This notched-ear characteristic was also found to be inherited as a simple autosomal dominant. Lush[1] reported the occurrence of moderate notches in the ears of Jersey heifers believed to be identical twins. One set of identical Jersey twin heifers collected by this station in 1954 was found to have moderately notched ears of the type described by Lush. Both Ruakura twins produced offspring with notched ears when mated to beef bulls

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†The terms "nicked" or "notched" were used in the U. S. Ayrshire Record and adopted by Yamane for descriptive purposes.

indicating a lack of breed specificity. It was not possible to trace the inheritance of these notches because of the haphazard breeding program followed in the commercial herd from which the identical twins came.

A third ear notch was reported in Norwegian cattle by Wriedt[4]. It was characterized by a peculiar notch in the tip of the ear on the margin of which was a papillary or wart-like outgrowth. Both ears in each affected animal were isomorphic, thickened and unusually hairy. The expression of the character varied; some ears being only 13 cm. compared with normal ear lengths of 17 to 19 cm. Wreidt described only two types of ears, namely, homozygous normal and heterozygous notched. The heterozygous notched-ear animals demonstrated marked phenotypic variability. One cow, heterozygous for the factor, showed no notch in the ears yet when mated to a homozygous normal male produced a notched-ear offspring. Other heterozygous animals had deep notches. This phenotypic variability and the heavily ribbed, hairy, wart-like margined appearance of the animals described by Wriedt suggests that these notches are due to a different genetic factor than that described by Yamane.

Description of Ear Notches Found in New Zealand

Visits were made to a number of purebred as well as grade Ayrshire herds in the North Island of New Zealand in an effort to trace the inheritance and gain a description of the reported aural abnormality. This natural notch occurred symetrically in both ears in each case investigated. Notches were found in Ayrshire × Hereford, Ayrshire × Jersey, and Ayrshire × Shorthorn as well as Ayrshire × Ayrshire matings, indicating they were not breed specific. Notched-ears were found in both sexes. The degree of notching varied greatly among the animals affected. Two major types were distinguished and the degree of notching remained constant throughout growth. The two animals observed to be severely notched were so conspicuously affected that the ears of these adult animals were as short as 4.5 to 6.0 inches compared to normal ear lengths of seven to nine inches. (Figure 13). Additionally, these ears were thicker than normal, showed a prominence of cartilage and muscle and a shape similar to a cut by stockmen for purposes of range herd identification which they term "swallow tail."

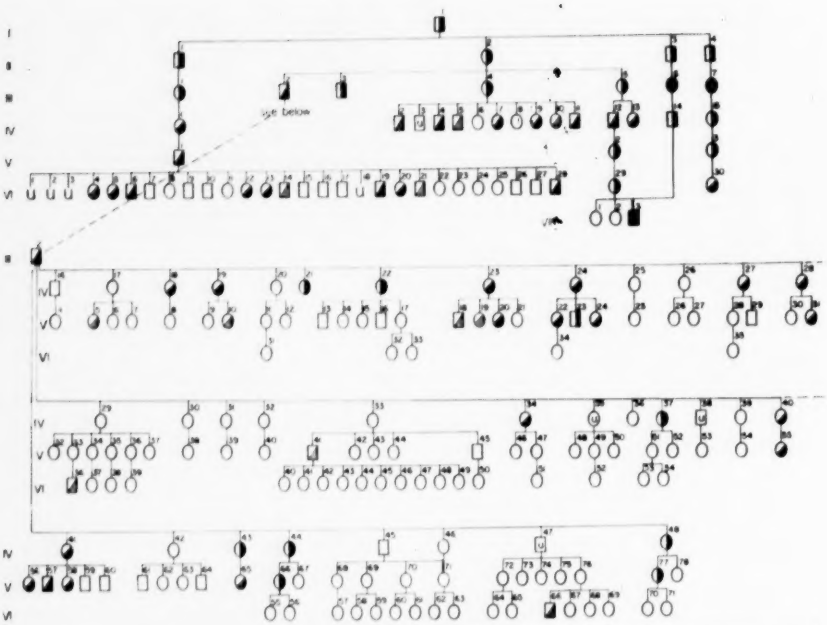
Greater variation in degree of notching was observed in animals of the less severely notched category due probably to the fact that much larger numbers of animals of this type were available for inspection. Some animals possessed only a small notch with none of the associative features characteristic of animals with moderately or severely notched ears. Others exhibited coarse hair, heavy cartilaginous tips, and ribbing, and a slab-like appearance due to a reduction in development of both anterior and posterior margins of each ear as the tip was approached.

Animals classified with moderate notches were much less similar to animals with large notches than those with small notches. It became apparent that arbitrarily defined categories for small and moderate notches were merely variations in the expressivity or penetrance of a single incomplete dominant factor while the severely notched ears were an expression of the homozygous dominant form.

Unlike Ohio, there is no discrimination against notched-ear cattle in New Zealand dairy shows. In fact two cows with notched ears are known to have won championships at major fairs. There was no significant difference in type or classification score between notched and normal-eared cows within the only sire groups with data available for study.

Mode of Inheritance

A geneological illustration (Figure 14) was compiled by examining living animals either personally or through reports of reliable livestockmen. Where there has been the least doubt as to the validity of data they have been omitted from the illustration and table or indicated by an appropriate symbol from



MODE OF INHERITANCE OF NOTCHED EARS

Figure 14

Mode of inheritance of notched ears, particularly in progeny of Ayrshire bulls I-1 and III-2. This is the most complete geneological history of the notched ear factor in New Zealand cattle of predominantly Ayshire breeding. The symbols used in the above pedigree are shown at the right.

CLASSIFICATION			SEX		
GROUP		NOTCH SIZE	MALE	FEMALE	UNKNOWN
KNOWN	NORMAL		□	○	
	ABNORMAL	LARGE	■	●	
		MODERATE	◪	◑	
		SMALL	◪	◑	
		UNSPECIFIED	◧	◑	
REPORTED ABNORMAL			◧	◑	
UNKNOWN			M	W	U

the legend. For ease of illustration animals have been coded rather than presented with full names and/or registration numbers.

Some of the notched-eared animals inspected were progeny of Eglington Mains Bright Light, an Ayrshire bull which was exported from Scotland to New Zealand. He carried the same herd prefix as the notched-ear bull who was exported to the United States from whence subsequent descendents of Eglington were exported to Japan and described by Yamane.

The most complete geneological history of the notched ear factor in New Zealand cattle of predominantly Ayrshire breeding appears in Figure 14. For ease of presentation the progeny of animal III-2 have been placed in three columns below collateral relatives. Attention is drawn to animals V-5, V-41, VI-36 and VI-66. It would appear that each of these animals represent notched-eared offspring of normal parents. In each case, however, the parent unrecorded in the illustration had notched ears. Both parents of VII-3, an animal with large ear notches, had notched ears. In all cases investigated this peripheral aural abnormality was found to be inherited only from a notched-ear parent. It would suggest that normal ears are homozygous and free of this abnormality. Furthermore since the only animal with large ear notches from which offspring where categorized left only notched-ear calves, it is hypothesized that large ear notches are homozygous and dominant. Animals with small or moderate ear notches were hypothesized heterozygotes indicating an incompletely dominant factor. It is suspected that this is the same genetic factor reported in Japan by Yamane.

This hypothesis was tested by analyzing the progeny of the bulls studied using the chi-square method. Animals according to hypothe-

sis, each the result of mating a normal and notched-ear animal, when mated to normal-eared animals should have notched-ear and normal progeny in the ratio of 1:1. The result of the analysis appears in Table I and substantiate with a "good fit" the hypothesis put forth.

Mode of Inheritance of Ear Notches

nn	*Nn* or *nN*	*NN*
Normal	small to moderate notch	large notch

As expected, after adjusting all records to a mature equivalent basis, the difference in milk production per lactation of 23.8 pounds of fat-corrected-milk (F.C.M.) in favor of notched-ear over normal-ear progeny of Bull III-2 was statistically insignificant.

Summary

Peripheral aural abnormalities appearing in New Zealand Ayrshire cattle have been described and the mode of inheritance traced.

The principal natural notch described occurred symmetrically in both ears. In homozygous recessive animals ears were normal while in homozygous dominant animals ears were deeply notched simulating a swallow tail livestock identification cut; were covered with coarse hair, and showed heavy cartilaginous tips and ribbing. In heterozygous animals this incomplete dominant character showed a varying degree of penetrance or expressivity varying from a simple small notch in the tip of the ear to a moderate notch associated with some coarsening of the hair and cartilage and a reduction in marginal tissue development toward the tip.

This factor was autosomal appearing in both sexes and was not breed specific.

As would be expected there was no relationship between the notched-ear factor and milk production or classification for type score.

Literature Cited

1. Lush, J. L. An hereditary notch in the ears of Jersey cattle. *Jour. Hered.* 13: 8-13. 1922.

2. Shrode, R. R. and J. L. Lush. Genetics of cattle. *Advances in Genetics* 1: 210-256. 1947.

3. Snyder, L. H. The Principles of Heredity. 3rd ed. Heath and Co. Boston. pp. 385. 1946.

4. Wriedt, C. Vererbliche Scharten an den Ohren des Rindes. *Zeitschrift fur Turzuchtung and Zuchtungsbiologie.* 3: 235-238. 1925.

5. Yamane, J. On the inheritance of an aural abnormality in the Ayrshire cattle. Hokkaido Imperial University, Sapporo. *Faculty Agri. Jour.* 6: 166-172. 1915.

TABLE I. Chi-suare analysis: Observed and expected numbers of normal and notched-ear cattle

Group	Obs.	Exp.	$(O-F)^2/E$
Notched-ear	35	37	0.108
Normal-ear	39	37	0.108
Total	74	74	0.216

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THE INHERITANCE OF COAT COLOR IN DOGS

DOG owners who have an occasional litter of varied-colored puppies as well as fanciers who have been trying to produce certain colors in their dogs, have been waiting for some one to present in simple and somewhat non-technical language the principles of the Mendelian-gene theory with evidence and examples in relation to the inheritance of coat colors of dogs.

Dr. Clarence C. Little has recently had such a book* published that may be readily understood by any dog breeder. He has adopted 10 series of alphabetical symbols for all the hereditary units or genes that may be applied to any and all breeds, which will make the genetics of coat colors more readily understandable in the 86 breeds of dogs that he has considered in his studies.

Dr. Little has been interested in the coat colors of mice, rabbits, guinea pigs and espe-

**The Inheritance of Coat Color in Dogs.* Clarence C. Little, Director Emeritus of the Jackson Memorial Laboratory, Bar Harbor, Maine. Comstock Publishing Co., associated with Cornell University Press, Ithaca, N. Y. 1957. 194 pp. $4.00.

cially of dogs since his early studies at Harvard University. In 1914 he demonstrated that the black and brown coats were controlled by different but related genes. He called the dominant gene for black (*B*) and its contrasting brown recessive mate (*b*). In the same year he found that another pair of genes (*cc*) caused the red and yellow coat colors in Pointer dogs, and which are now known to produce the apricot coat colors in Poodles. A few years later he demonstrated that another pair of genes (*dd*) caused a clumping together of the pigment granules in small masses in the hairs, so that when the light struck them in the black coats, a blue-color effect was produced, the Maltese blue. When the clumping occurred in brown hairs a different shade of brown was produced throughout the entire coat. The clumping forms early and may be readily seen under a microscope in the coats of new-born puppies. In 1919 he collaborated with E. E. Jones in an extensive study of the inheritance of the various color patterns of the Great Danes.

From 1922-1929 he became active in executive and administrative work and was president of the University of Maine and of the University of Michigan. In 1929 he was appointed Director of the Jackson Memorial Laboratory at Bar Harbor, Maine, where an intensive program has been carried on in the study of the inheritance of the susceptibility and non-susceptibility of cancer, mainly in mice, and the modification of germ plasm.

A few years ago the Rockefeller Foundation assigned a large grant to the Bar Harbor Laboratory for the study of the genetic behavior patterns in dogs. A colony of 200-225 dogs of 28 breeds was gradually established. These dogs have been bred and crossbred and have produced about 4100 puppies. Not only the behavior patterns have been studied but also the coat colors. In addition to these studies of the laboratory puppies, more than 20,000 kennel reports (as published in 1953) from various reliable dog breeders were received and analyzed in regard to coat colors of the puppies. A summary of the tables in the book shows a total of 37,187 puppies used in the studies of coat colors.

From these studies, some of which were very extensive as of the Cocker Spaniels, Great Danes and German Shepherds, and others that were meager, owing to the small amount of information available, Dr. Little has selected the 10 sets of alphabetical symbols for the genes that will aid in explaining the relation of a puppy's coat color to its parents in the many breeds studied and, furthermore, may aid in the prediction of the possible colors in future litters.

Recently, Dr. Little has demonstrated the presence of an incomplete dominant-graying gene (*G*) in a few breeds. A single gene produces only a slight graying and bluing effect in the Kerry Blue Terriers but in the black Poodles it may not produce the silvering effects for several years, if ever. When, however, two of these dominant genes (*GG*) are present the beautiful gray and blue of the Kerry Blues develop and the lovely silver coats of the black Poodles develop during the first few months of their lives. This discovery will be of great interest to Poodle fanciers to know the reason why the puppies that carry a dominant silver gene do not readily clear to silver.

The explanation of the production of the two kinds of white coats will also be of much interest to many people. Dr. Little shows that by a certain assortment of genes in some breeds, whites, either with or without a cream tinge, may be produced. In 29 of the spotted breeds, however, white coats may appear when a pair of "vanishing genes" (*s*^{w}*s*^{w}) are present which prevent colors from being developed, but which, in certain later matings, may reappear in future generations.

These studies, directed by an eminent geneticist, on the inheritance of coat colors in about two-thirds of the known breeds of dogs, is undoubtedly the most extensive study that has ever been made. It does not claim to be complete but suggestions are made for future additions and corrections. All who are interested in the color of dogs are under a deep obligation to Dr. Little for simplifying the presentation of the Mendelian theory of inheritance and for the list of proven and suggested genes of each breed studied.

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ORIGIN AND INHERITANCE OF D_2 SMOOTHNESS IN UPLAND COTTON

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UPLAND cotton and many of its nearest relatives are normally hairy; they belong to the species known as *Gossypium hirsutum* L. A characteristic, which is called D_2 Smoothness, has been transferred from a wild, lintless, glabrous American species to Upland cotton. Plants homozygous for D_2 Smoothness lack hairs on the stems, leaves and bracts (Figure 15). D_2 Smoothness appears to be a promising new plant property that can help solve the trash and lint-cleaning problems of mechanically harvested cotton[2]. This report outlines the transfer of this smoothness to Upland cotton, presents genetic data, and proposes a gene symbol.

SMOOTH AND HAIRY COTTON

Figure 15

The stem section at the left shows the hairless condition produced by the dominant gene *Sm*. The stem section at the right shows the typical long hairs of Upland cotton produced by the recessive gene *sm*.

Origin of D_2 Smoothness

Scions of a pentaploid cotton hybrid (Texas Hybrid 5207) involving a Deltapine variety of Upland cotton ($2n = 52$) and the lintless, diploid, photoperiodic, American species *G. armourianum* Kearney ($2n = 26$) were obtained and grafted in 1948. Pentaploids of this type do not produce seeds but they are pollen-fertile. Pollen of the pentaploid hybrid was used to make crosses on Z106, a doubled haploid of a Stoneville variety of Upland cotton. A progeny from crossing Z106 ($2n = 52$) with the pentaploid hybrid ($2n = 65$) was grown and the resulting plants self-pollinated. Fifty-two S_1 progenies from individual plants were grown in the field during 1951. Six of the progenies had plants that were extremely smooth or lacking in plant hairs. Most of these smooth plants were very small, late or unproductive. Many did not flower. The plants in one row were outstanding; 8 of the 9 plants in this row were extremely smooth. One plant was smooth, early, vigorous, had many flowers with good pollen, set many bolls, and had fairly acceptable lint. This plant was backcrossed to Z106 and was also crossed with M8948, a doubled haploid of a Deltapine type of cotton.

Some of the smooth plants of the first backcross generation to Z106 were again

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backcrossed to Z106. Two backcrosses were made each year by crossing heterozygous smooth plants with Z106 as the recurrent parent and, in a separate line of endeavor, by crossing smooth plants with M8948 as the recurrent parent. Seed stocks homozygous for D_2 Smoothness produced by selfing after four backcrosses to Z106 and after three backcrosses to M8948 were officially released to state, federal and commercial breeders and geneticists during 1954.

Inheritance of D_2 Smoothness

Table I summarizes segregation results that were obtained from three different F_2 generations and from one backcross generation, involving 4918 plants. Assuming that D_2 Smoothness is due to one dominant gene, the value of P for the chi-square values varied from 0.10-.20 to 0.30-0.50. These results indicate that D_2 Smoothness is due to one dominant gene. It is very difficult to distinguish between plants that are homozygous and plants that are heterozygous for D_2 Smoothness. Therefore, the gene that determines this smoothness is almost completely dominant. Crosses with some cotton varieties produce heterozygous plants with varying degrees of rudimentary plant hairs, suggesting that the effect of this major gene for plant smoothness is modified by minor genes.

The symbol *Sm* is proposed for the dominant gene which causes D_2 Smoothness, with *sm* being the ordinary hairy or non-smooth gene at the same locus in *G. hirsutum* L. Since it was transferred from an American D species, the locus *Sm* must be in the D subgenome and not in the A subgenome of Upland cotton.

Discussion

Fibers on cotton seeds are elongated extensions of single epidermal cells on the outer integument of the ovule. Since the epidermis of the ovules and the epidermis of the stems and leaves are the same tissue, it might be expected that genes with pleiotropic effects on lint and on the hairs of stems and leaves might be found in cotton.

Simpson[3] found a dominant gene in Upland cotton which caused very hairy cotton plants; these hairy plants had reduced lint length. Hutchinson and Gadkari[1] found that two of four genes that produce lintless Asiatic cottons also cause glabrous plant body as a pleiotropic effect.

The presence of the gene for D_2 Smoothness results in a glabrous plant body in mature plants but it does not materially change the amount of lint by weight nor does it greatly modify lint properties. Young plants with D_2 Smoothness do have some hairs, but the mature stems, leaves and bracts become glabrous. The hairs apparently fall off.

Summary

D_2 Smoothness is due to a single dominant gene which has been transferred from *G. armourianum* Kearney—a wild, lintless, glabrous, photoperiodic, American species—to Upland cotton. This dominant gene appears to be a promising new plant property that can help solve the trash and lint-cleaning problems of mechanically harvested cotton. The symbol *Sm* is proposed for this gene, which should be in the D subgenome of Upland cotton because it was transferred from an American D species.

Literature Cited

1. Hutchinson, J. B. and P. D. Gadkari. Genetics of lintlessness in Asiatic cottons. *Jour. Genet.* 35:161. 1937.

2. Lund, Zane F. and James R. Meyer. Development of smooth leaf cotton. *Miss. Agri. Expt. Sta. Service Sheet* 428. 1954.

3. Simpson, D. M. Fuzzy leaf in cotton and its association with short lint. *Jour. Hered.* 38:153. 1947.

TABLE I. Segregations obtained in backcross and selfed backcross populations from Upland cotton plants heterozygous for D_2 Smoothness

Population	Plant segregation		Segregation ratio	χ^2	P
	Smooth	Hairy			
(A) *Selfed progeny from:*					
2nd backcross to M8948	412	157	3:1	2.039	.20-.10
2nd backcross to Z106	1730	543	3:1	1.496	.30-.20
3rd backcross to Z106	965	294	3:1	1.824	.20-.10
(B) *Backcross progeny from:*					
4th backcross to Z106	419	398	1:1	0.540	.50-.30

LORDOSCOLIOSIS IN THE TROPICAL FISH

Panchax lineatus

KENICHI KENNETH HISAOKA*

LORDOSCOLIOTIC MALE

Figure 16

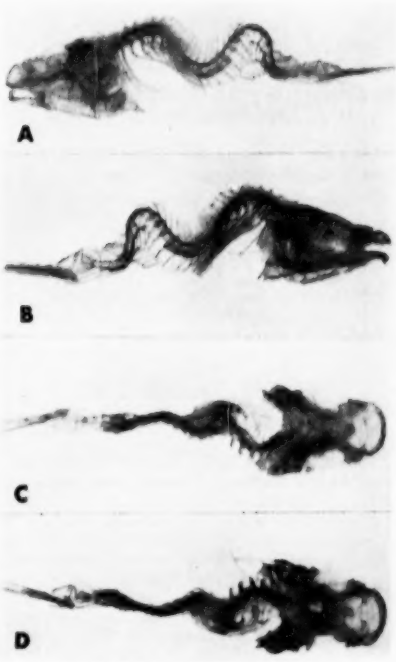
Lordoscoliotic male specimen of *Panchax lineatus* at two months of age. Total length was 2.7 cm., (3.2×).

ALTHOUGH some genetical studies have been made on lordotic tropical fishes[1-4] less attention has been directed to the actual study of the abnormality itself. In many instances, the fishes were evidently assumed to be lordotic on the basis of external features only. In this report, some morphological aspects of the vertebral column of a male lordotic specimen of *Panchax lineatus* are presented.

One male specimen of *Panchax lineatus* which possessed a pronounced dorsoventral curvature of the body was obtained during the latter part of the summer of 1956. Although this abnormality was present at birth, it did not interfere in any way with swimming movements or feeding habits.

Although it was the intention of the author to raise this fish to maturity for genetical studies, it died suddenly at three months of age. The fish was cleared and then stained with alizarin red.

An examination of the external features of the living specimen of *Panchax lineatus* revealed only a dorso-ventral curvature of the body as shown in Figure 16. As may be seen in this figure, the body and tail were relatively transparent



SKELETAL ASPECTS OF LORDOSCOLIOSIS

Figure 17

A-D Shows the left side, right side, ventral and dorsal views respectively of the vertebral column stained with alizarin red at the age of three months. Total length was 3.2 cm., (2×).

and the vertebral column appeared to follow a corresponding dorso-ventral bend of the body. Without further study, such a fish would have been described as being lordotic. However, a study of the skeleton of this specimen demonstrated that in addition to the dorso-ventral curvatures (Figure 17 *A* and *B*) there were also lateral curvatures which were very pronounced (Figures 17 *C* and *D*). The greatest lateral curva-

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ture of the vertebral column occurred immediately posterior to the skull where there was a marked convexity to the left side of the body. This was immediately followed by a compensatory curve which was directed to the opposite side. Although the vertebral column itself exhibited rather extreme scoliotic characteristics, lateral curvatures were not evident in the living fish. Nevertheless, the abnormality is actually lordoscoliosis.

It is apparent from this study that the actual curvature of the vertebral column does not necessarily correspond to the external contours of a lordotic fish. Therefore, specimens of this type should always be studied by sectioning, staining or by X-ray before the nature of the abnormality is assumed.

Literature Cited

1. Aida, T. Further genetical studies of *Aplocheilus latipes*. *Genetics* 15:1-16. 1930.
2. Goodrich, H. B., R. W. Harrison, N. D. Josephson and J. P. Trinkaus. Three genes in *Lebistes reticulatus*. *Genetics* 28:75. 1943.
3. Rosenthal, H. L. and R. S. Rosenthal. Lordosis, a mutation in the guppy. *Jour. Hered.* 41:217-218. 1950.
4. Rosenthal, H. L. Studies on semi-lethalism in the male lordotic guppy. *Jour. Hered.* 42:257-259. 1951.

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Closing Date for Research Proposals—National Science Foundation

The Division of Biological and Medical Sciences of the National Science Foundation announces that the next closing date for receipt of research proposals in the life sciences is January 15, 1958. Proposals received prior to that date will be reviewed at the Winter meetings of the Foundation's Advisory panels and disposition will be made approximately four months following the closing date. Proposals received after the January 15, 1958, closing date will be reviewed following the Spring closing date of May 15, 1958.

In addition to funds for the support of basic research in the life sciences, limited funds will be available during the current fiscal year for the support of research facilities and programs at biological field stations.

Inquiries should be addressed to National Science Foundation, Washington 25, D. C.

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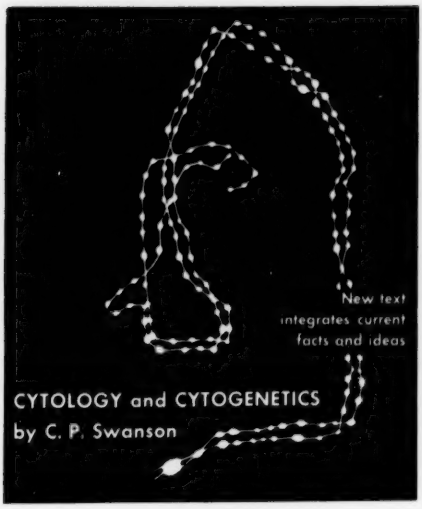
Corrigendum

An error has been found in Table I of my paper, "Cytological Studies in Saccharum and Allied Genera. IV. Hybrids from *S. officinarum* ($2n = 80$) × *S. spontaneum* ($2n = 96$)": *Jour. Hered.* 48:141-145. 1957. The following correction should be noted:

In Table I the chromosome numbers of hybrids P. B. 52-1-1, P. B. 52-1-4 and P. B. 52-1-7 should be interpreted as $2n + n$.

Sam Price

Hawaiian Sugar Planters Association, Honolulu



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